PHYTOPATHOLOGY

VOLUME 18 VOLUME 6

JUNE, 1928

BLIGHT-RESISTANT POTATOES

DONALD REDDICK

The primary purpose of this article is to make a second report of the progress that has been made in the development of a potato (*Solanum tuberosum* L.) that is resistant to the disease known as late blight or, more commonly, blight [caused by *Phytophthora infestans* (Mont.) deBy.]. A short discussion of biologic specialization and of the origin of the parasite is added.

Since the appearance of the first brief report on this subject by the writer (41), extensive reports on a similar endeavor instituted by Broili (11) in Berlin have appeared. K. O. Müller (35), in particular, has summarized the early work in breeding for disease resistance and especially for blight resistance, in potatoes; and Appel (4) has published a more popular account of the same sort. In view of the fact that the work is scarcely known in the United States, these authors and others may be pardoned for not including in their reviews a reference to one very important effort, and perhaps the first one, to overcome blight by breeding methods. Almost as soon as blight became a problem in the United States, Chauncey E. Goodrich (19), of Utica, New York, began work which extended over a period of nearly 20 years on the "regeneration" of the potato. Although Goodrich never admitted the causal relation of *Phytophthora infestans* to the blight, his stand on this subject coincided with that of nearly every other person in the world who had an opinion on the etiology of blight. If this one error is overlooked it will be found that Goodrich's observations on the occurrence and spread of blight, his description of the disease in all its phases, and his observations on the attendant conditions of an outbreak are extremely accurate in every detail. Goodrich made a very positive contribution to potato culture by the introduction of new "blood" into an old line; a contribution which is fully acknowledged by Salaman (45, p. 7) in his recent book, "Potato Varieties." Furthermore, Goodrich's contribution was particularly important in that he showed definitely that races of potatoes exist

which are highly resistant to the disease, that these "wild" potatoes can be ameliorated by hybridization with the commonly cultivated sorts, and that resistance is transferred to a part of the progeny. While it is not perfectly certain, there are strong indications that the variety Evergreen, which is now cultivated to a certain extent and which is so resistant to blight that a good crop can be secured even in a year of severe blight, is one of Goodrich's hybrids.

Evergreen has been sent to the writer repeatedly under the name of "blightproof." It is also the variety which is grown in New Jersey under the name "Red Skin," and it is also the variety which Jehle (25) has recently recommended to Maryland potato growers as a substitute for McCormick. Jehle's very recent record (26) of as high as 25 per cent of Phytophthora tuber-rot in "Red Skin" indicates that there may be several closely related varieties known by this name. So much rot of tubers has not been found at Ithaca where tests have been made on several lots, including one sample from Doctor Jehle.

Evergreen is well named in that the variety is highly tolerant of dry weather and forms a very large green vine when such varieties as Green Mountain or even Rural are severely injured. It is by no means immune to *Phytophthora*. In a four-group classification of plants in which 4 represents as complete absence of resistance as is exhibited by Green Mountain, Evergreen is usually listed at 3, although some strains have been tested in 1927 which may be listed at 2. The variety often develops seedballs when grown in the field with other varieties, but all attempts (several hundred) to self the variety in greenhouse or field have failed. It seems to be completely male sterile, and no pollen grains that appeared to be normal ever have been seen. Viable pollen of several varieties has been tested on Evergreen, and seedballs have been produced. When pollen of a homozygous variety (Ekishirazu) is used, the first generation shows such a heterogeneity that Evergreen is certainly heterozygous. The genetics of Phytophthora resistance is difficult because homozygocity is impossible to obtain with a large number of varieties which are male sterile. Even in certain wild species of *Solanum* an incompatibility exists which makes selfing very difficult or even impossible. Genetical work in collaboration with C. H. Myers, of Cornell University, is in progress but will not be reported at this time further than to say that resistance to *Phytophthora* is a heritable character, at least in certain instances, as will be apparent from subsequent remarks.

The prime requisite in breeding for resistance to *Phytophthora* is, of course, the existence of a resistant stock. Fortunately this is at hand in Ekishirazu (blight unknown or blight proof), a variety which was described by Ito (24).

It would be possible to use Ekishirazu directly in commercial culture, at least in the Northern States and Canada, if the variety were suitable for the purpose. Unfortunately it is not suitable. The variety has not made a good growth at Ithaca, New York, in any of the five years that it has been under test. The plants in the field are very short and prostrate, although they have been grown in the greenhouse simultaneously and commonly attain a height of 2 meters or more, and the tubers rarely have reached marketable size. In addition, the rough, scaly skin is objectionable, and the flavor is not all that could be desired in a good commercial sort. The resistance of the variety to blight is its most commendable character. This resistance is not absolute. It is always possible in the greenhouse to secure infection on the very young leaves at the tip of the stem. The tip of the stem itself becomes infected too but the lesion produced rarely extends as far as 2 centimeters down the stem. Lesions also may appear on older leaflets, but these usually are not more than one millimeter in diameter although they may attain a diameter of 3 mm. Leaf lesions have not been observed in field-grown plants, and the stem-tip lesions are very uncommon. Furthermore, the fungus rarely fruits on these lesions even when affected parts are placed in a humid chamber. Aerial mycelium is developed under such conditions, and in some instances sporangia have been found. When a number of lesions develop on a single leaflet or when one or two lesions develop on several leaflets, the whole leaf is very likely to turn yellow and fall from the plant. The incubation period is practically identical with that on such a susceptible variety as Green Mountain; at most, it is only a few hours longer on Ekishirazu. The variety possesses a very high degree of resistance, and if it were grown commercially at Ithaca there would be no loss from blight even though susceptible varieties proved a total loss when grown alongside. Plants approximating this condition are given a rating of 1 in tests for resistance. A plant that can be rated 0 is still sought.

Ekishirazu, fortunately, flowers freely, is self fertile, and produces seeds very readily. When flowers of this variety are selfed they do not always set fruit, but when the pollen of Ekishirazu is used upon any one of a number of commercial sorts a set of fruit is almost certain to result. Seedlings of selfed Ekishirazu, when grown and inoculated with *P. infestans* in a flat in the greenhouse, were somewhat susceptible. All of the plants at the time of inoculation had four to six leaves, all of which were of juvenile (entire) form. A few plants were killed. Those remaining, whether affected or not, were moved to the field. The blight did not spread on any of the plants, nor did the plants become affected when inoculations (both artificial and natural) were made on them later in the summer.

Evergreen and Ekishirazu have been described in some detail not only because they are not widely known but also because hybrids of them have

yielded the largest percentages of resistant plants of any of the combinations thus far attempted. A few other varities may be mentioned briefly for certain characters that have made them useful in blight-resistance work. Irish Cobbler, as a female parent, when crossed with Ekishirazu has yielded a goodly percentage of promising plants. This variety, it may be noted, traces back through Early Rose and Garnet Chili to Goodrich's Rough Purple Chile. Green Mountain, the various Rurals, American Giant, Up-to-Date and the like, when used as female parents, have proved very disappointing. Some varieties which were secured in the Vosges of Alsace in 1924 and which were selected because of their very marked resistance to blight (single green plants standing in potato fields that had been completely destroyed by *Phytophthora infestans*) give evidence that they may prove valuable as foundation stock for breeding purposes. The names of these varieties can not be ascertained. One of them is known locally as Steinthaler, but this is only the name of the valley in which it grows and is not a recognized varietal name in European literature. Another is called Halbfrüh, but here again the name is one to indicate date of maturity and is not a known varietal name. These plants grew very satisfactorily at Ithaca in the summer of 1925 and of 1926, but in 1927 the dry and warm (at times) weather affected them as seriously as, or more so than, Green Mountain, a variety which is markedly affected by such conditions. Their usefulness therefore remains problematical.

Early in the work, first generation hybrids were tested in the seedling condition for Phytophthora resistance. Practically all the seedlings became affected, and it was tentatively assumed that if resistance were a heritable character at all it must be transmitted as a recessive. When the seedlings of Ekishirazu showed infection, this opinion had to be revised; and it is now known that it is worth while to make field tests on hybrids of the first generation. In some cases this is highly important since the hybrids may prove to be male sterile and in such cases a second seminal generation can not be obtained. It is worthy of note in passing, however, that plants on which no seedballs have set after several hundred trials may for no apparent reason produce one or more flowers which self readily and which produce good seeds. Most of the tests on which this report is based have been made with second generation hybrids, although a few involve first generation hybrids and a few others those of the third generation.

Thanks to the work of Melhus (33), and now to the extensive work by Vowinckle(51), the method of securing infection with *P. infestans* is well understood. No difficulty is involved in the greenhouse where external conditions can be controlled. But since greenhouse tests with seedlings are not reliable, and since several thousand plants have been tested each year, it has

been easier to run the tests in the field. Under field conditions it is not always so easy to secure infection. It happens that, during the three years 1925 to 1927, inclusive, seven or eight separate inoculations have been made each year before the blight became satisfactorily abundant in the field so that a uniform natural infection could be expected to occur. Thus far the classification of plants into categories of resistance has been based upon a natural spread of the disease from alternate rows of susceptible plants which became infected following artificial inoculation. It is perhaps possible to make eliminations after an artificial inoculation but there is always some risk that really desirable plants will be discarded. It has usually happened that the field inoculations have been made just at dusk and there has been no time to regulate the number of swarm-spores that were applied to each plant nor to see more than that each plant received some of the inoculum. Under such conditions certain leaves of a plant are likely to receive an excessive number of viable spores and to be destroyed by the fusing of very many small lesions. It also happens frequently that the progress of the lesion on a leaflet and the abundant production of conidia indicate that the plant ultimately will be classed as completely susceptible. Subsequent events, however, show that only the affected leaflet is involved and that the leaflet either becomes dry or perhaps the whole leaf yellows and falls. The fungus does not spread down the midrib of the leaflet and involve the other leaflets nor the stem. In other cases the number of penetrations on a resistant plant is very small. Intermingled leaves of a susceptible plant may show dozens of separate infections, whereas only a few infections occur on the resistant plant.

In 1925 the work was cut short by frost. Every plant that showed indication of resistance was saved for further test. In 1926 conditions were ideal for the test: a better year for such work never can be hoped for. During the month of August there were 26 days recorded as cloudy or partly cloudy, with precipitation on 20 days and with minimum temperatures of 13° C. or lower on eight nights as follows: 9, 10, 21, 22, 26, 28, 30, 31. In September there were 23 cloudy or partly cloudy days, there was precipitation on 15 days, and there were 21 nights with minimum temperatures of 13° C. or lower. The plants were not killed by frost until the night of October 16. Of the 16 days, 14 were cloudy or partly cloudy, there was precipitation on 12 days and the minimum temperature was at or below 13° C. on 13 nights. In 1927 the conditions were not so good and were complicated by the fact that many lots of plants were injured by extreme drouth. So far as commercial production is concerned, however, the season made possible the elimination of drouth-susceptible plants. The last artificial inoculation of 1927, nevertheless, was made under ideal conditions—a great quantity of

swarmspores in condition to germinate at once, a very fine misty rain setting in before the inoculation was completed, and the night one of fog and mist. Every plant used as a check became infected, and this meant every other row in a field of more than two acres. The natural infections occurred subsequently, one of which was extremely heavy and another one not so heavy, the latter due in part to the fact that some of the susceptible plants were already dead, thus reducing the quantity of inoculum. The yield in 1927 was very unsatisfactory but this condition existed in most fields in the vicinity and is attributable to dry weather. Each year most of the plants that graded 1 or 2 in a scale of 4 were saved for further test, but plants exhibiting obviously undesirable characters such as dwarfing, frenching, evidence of extreme drouth injury and the like, either in vine or tuber, were discarded at harvest time. The suggestions of Stuart (47) about the kinds of seedling tubers that are worth propagating beyond the first clonal generation were kept in mind but were not applied at all rigidly. It was desired to carry through several generations a considerable number of lots that exhibited high resistance in order to see whether any or all might lose their resistance in the way that de Candolle (14, 15) reports for *Solanum verrucosum* Schlecht.

There exist nearly 300 separate lots of plants for most of which there is a definite record of blight resistance for the two consecutive years 1926 and 1927 with the general record that these plants exhibited decided resistance (grade 1 or 2) in 1925. Upon tabulation it appears that 43 lots of plants have been given a grade of 1 for the two years and 116 have been given the grade 2. Eighty-six lots that were marked grade 2 in 1926, a highly favorable season for blight, were marked grade 1 in 1927, a less favorable year for blight. On the other hand 12 plants of grade 1 in 1926 dropped to grade 2 in 1927, and 19 plants of grade 2 dropped to grades 3 or 4. These records will be supplemented by behaviors in 1928 and will be further supplemented by 272 plants which have been graded 1, and 289 that have been graded 2 in 1927, as well as by over 2,000 new lots which are to go into the field for the first time in 1928.

From the foregoing record it appears fairly well established that resistance to *Phytophthora infestans* in these various lots is rather definitely fixed. In fact it is entirely conceivable that their resistance does not vary at all but that the writer's ability to classify degrees of resistance accounts for all of the variation. Such a classification is much less definite than, for example, one involving the amount of rust on a blade of wheat. After passing judgment upon a few hundred plants one acquires a certain discrimination which is useful for the remaining plants. At the end one must return to the first hundred or more plants in order to give them a fair and comparable rating.

Certain anomalies have existed throughout the work. Occasional instances have been noted in which a certain lot of plants has been classed 1 throughout a whole season, only to drop to 3 or 4 the next year. The reverse of this has not been observed because a plant rated 3 or 4 has been discarded. While it would be easy to say that such plants have lost resistance, it would be difficult to prove it, and for the present the statement that the plants simply escaped infection fortuitously is just as likely an explanation.

Another almost anomalous condition which is evident only in the field notes is one in which a plant is recorded as showing resistance 3 and after a few days is marked up to 2 or even to 1. The rapidity with which a few leaflets are being destroyed leads to the assumption that the whole plant will be killed, whereas these few leaflets very soon die and the number of new infections from period to period is so small that careful examination is required to find that the disease exists there at all.

Still another condition has been observed which is very interesting. Certain very large plants reached a condition in late summer in which the lower leaves were so much shaded that they turned yellow and eventually dropped from the plant. Following a particularly heavy natural infection in 1926 it was found that the green foliage of some such plants was entirely free from lesions or, in other cases, at the most, showed only very small spots about 1 mm. in diameter. The senescent leaves, however, were attacked and the fungus could be found fruiting upon them. No definite lesion was evident, and the sporangiophores were so scattered that their presence would not have been suspected except for the fact that each one bore a droplet of dew. This condition is of interest from both the theoretical and from the practical standpoint and is one that must receive further study. Vowinckle (51, p. 616) presents data for a susceptible variety which anticipate this result in that he found susceptibility increasing with the age of the host and a higher susceptibility of the basal leaves of a plant than of those higher up. Miss deBruyn (12) also found the same condition existing when susceptible varieties were inoculated in the field, and thus differs with Pethybridge (39), who concluded that there was no material difference in susceptibility of plants in different stages of development.

Another condition that has been observed, clearly not an anomaly, is that foliage may show a high degree of resistance while tubers of the same plant exhibit little or no resistance. This is the antithesis of the condition described by Miss Löhnis (32) for the variety Bravo. No systematic test of tubers for resistance has been made as yet, and this mention of tuber susceptibility is based only on those occurrences of natural infection that have been encountered. The presence of affected Green Mountain foliage,

often intermingled with the healthy foliage of resistant plants, furnished abundant inoculum, and the destruction of Green Mountain tubers from rot has ranged from 50 to 100 per cent. A hill of Green Mountain that was free from tuber rot scarcely ever has been found, and when one such was found it was usually evident that the plant had been killed before blight became prevalent in the field. Since this condition was general throughout the field and since the number of lots of resistant plants showing tuber infection has been small, even under conditions extremely favorable for Phytophthora rot, it is assumed, tentatively, that resistance to infection in tubers is often coupled with resistance in foliage. From the practical standpoint it is obvious that tuber susceptibility is not a matter of great importance provided the foliage shows a resistance of grade 1, *i.e.*, so resistant that the pathogene is not able to perpetuate itself.

The susceptibility of fruits is of interest too. Not many records are available, but those that are show that seedballs on susceptible varieties are very susceptible. Those on Evergreen (resistance grade 3) apparently are as susceptible as on Green Mountain (grade 4), and, interestingly enough, those half-dozen or more seedballs which were produced in the field on a plant of grade 2 seemed to have no resistance at all. Unfortunately no records are available on the condition exhibited by seedballs on plants of grade 1. It is entirely possible that they have no resistance either, just as Lesley (30) has found in *Solanum edinense*, in which case a perfect transition would be effected to the tomato and finally to the egg-plant. This will be brought up again in another connection.

Very few, if any, of the various lots of the hybrids are exactly alike. All sorts of variations exist and it should prove possible to select for a considerable range of requirements. There are even indications that early sorts may be found, although this was not anticipated and as yet no adequate test has been made. Likewise no cooking tests have been possible with the resistant plants. A great many of the susceptible sorts have been given a cursory test and in the vast majority of cases the hybrids have proved of better quality and flavor than their resistant parent. Records on conditions after storage are available but are not particularly significant as yet because of the small amount of tubers available for test.

It is the intention to select several of the more promising sorts for rapid increase so that samples may be distributed widely for testing under a great variety of conditions. The plan also includes a test of certain of the most resistant sorts in a number of foreign countries, not so much with the idea that the plants may prove adaptable to local use in commerce as to find out whether *Phytophthora infestans* is biologically specialized. The advantage of investigating this problem under identical conditions of growth of the

host is appreciated, but on the other hand the difficulties of securing the necessary material for tests at Ithaca are too well known to require enumeration.

Likewise there is a positive advantage in conducting such tests under dissimilar conditions. Until the discovery of the existence of biologic specialization, it was conventional to assume that differences in behavior of a given host to a specific disease under widely differing conditions was occasioned by the effect of the environment on the host. Biologic specialization has been found to furnish a more satisfactory explanation of some of these cases but it does not follow by any means that it is always the explanation. One has only to inoculate potato plants with *P. infestans* under glass and in the field to know that environmental factors do make a difference in the amount of disease that results. Persons who are interested in securing plants or in extending this investigation in any possible manner are invited to make their wishes known.

BIOLOGICAL SPECIALIZATION

In the absence of definite information on the subject, it has been assumed for the purpose of this work that biological specialization in *Phytophthora infestans* does not exist. The assumption, however, has not been made without some foundation in fact. Indeed there has been very little found out about this organism which would indicate the existence of high specialization. The difficulty of bringing the fungus into pure culture on an artificial medium has been made the basis for an assumption of approximate obligate parasitism and a high specialization which the recent work of Miss deBruyn (13) has done much to dispel.

Likewise the infection of senescent foliage recorded earlier in this paper points to a rather low order of parasitism, as does the fact that the fungus kills its host outright. In the same way the wide host range of *P. infestans* argues against a high degree of specialization. The fungus is reported to occur on several different Solanaceae, some of them rather far removed from the Tuberarium group, and two of them (*Schizanthus* and *Anthocercis*) so far removed that in older taxonomic works they are to be found listed with the Scrophulariaceae. Some of the species affected are: *S. utile* Klotzsch (Münter, 37); *S. verrucosum* Schlecht. (de Candolle, 15); *Solanum dulcamara* L. and *Schizanthus grahami* (deBary, 5); *Anthocercis viscosa* (Berkeley according to deBary, 5); leaves and fruit of *S. muricatum, S. caripense* Kunth, and *Petunia* hybrids (Lagerheim, 29); *Lycopersicum esculentum* (Payen, 38 and Berkeley, 7); *S. maglia* Molina (Darwin ?, 17 and Sutton, 48); *S. demissum* Lindl. and *S. cardiophyllum* Lindl. (Lindley, 31); *S. edinense* Berthault (Salaman, 44); *S. aviculare* (Berg, 6); *Lycium*

halimifolium, L. turcomanicum, Solanum nigrum, Physalis alkekengi, Hyoscyamus niger (Vowinckle, 51), the last four only on detached leaves. In this laboratory infection has been secured on *S. commersonii* Dunal (3 samples from coastal plain of Southeastern South America); *S. maglia* from the island of Chiloé, Chili; *S. fendleri* Gray and *S. jamesii* Torrey from Southwestern United States. *S. dulcamara* has become infected repeatedly by inoculating with the fungus taken from cultivated potato. Just as described by deBary (5), the lesion produced is very small, and the leaf soon yellows and falls, but *P. infestans* is present abundantly and fruits freely on affected leaves which are put in a humid place. *Solanum nigrum, S. carolinense, Physalis alkekengi, Hyoscyamus niger* and some other solanaceous hosts of uncertain identity did not become infected either in greenhouse or field. No trials were made with detached leaves. Leaves of *S. melongenum* also have become infected when the host has been weakened by exposure for three days in a humid chamber with weak light. Haskell (21) has reported the occurrence of lesions on the calyces and fruits of this host which he attributes on strong circumstantial evidence to the *Phytophthora* from potato. Salaman (44) reports that *S. edinense* was highly resistant in his cultures for 20 years but that suddenly, with the resumption of the sexual process of reproduction, the species became susceptible. This seems to be a revival and extension of the "principle of compensation" which was defended by de Candolle (15) years ago, and which has been invoked subsequently on many occasions, particularly by Jones (28), who speaks frequently of the "critical period" in the life of the plant. Pethybridge (39) has done much to dispel this idea of a critical period and the weakening of resistance at the time of flowering and of tuber formation. The circumstance reported by Salaman certainly suggests the introduction of a new biologic form of the fungus into his cultures, but there seems to be no way of testing this point now unless *S. edinense* of the same clonal line can be found which has not yet resumed sexual activity.

It may be contended that a fungus does not have to be a highly specialized parasite in order to exhibit the phenomenon of biologic specialization. That this may be true is indicated by the recent and extensive work of Berg (6). Berg's experiments are so numerous and so comprehensive that his conclusions seem fully justified. Berg used in his experiments the tomato variety Stone, a fact communicated by him in correspondence. For the purposes of his work the variety name is not important, but in comparing his work with that of Bondartseva-Monteverde (8, 9) one is led to the conclusion that the latter worked only with that fungus which Berg would call the potato strain. Nothing in Bondartseva's paper can be taken to indicate that she was dealing with specialized forms. While it seems im-

possible to place any other interpretation on Berg's experimental data than he does himself, nevertheless the following sentence in his "Field Observations" seems to constitute a paradox. His statement is (p. 27): "In instances where the early development of the blight was kept under close observation, the first signs of tomato blight were found on the plants adjacent to potato plants that were heavily infected with *P. infestans.*" In his summary he makes the following statement which is even more significant: "In all cases observed under natural conditions, potato late blight appeared earlier in the season than the tomato late blight and wherever tomato late blight was found potato late blight also occurred in close proximity."

An epiphytotic of Phytophthora blight and fruit rot of tomato has occurred in the southern coastal counties of California in the autumns of 1927 and—to a more limited extent—1926 as reported in personal correspondence with G. B. Ramsey and I. C. Jagger. The latter upon request has made some cursory inquiries and upon rather satisfactory circumstantial evidence suggests that the same organism is probably causing the blight of both potatoes and tomatoes in this region. Some comparisons of the tomato fungus from California with the potato fungus from Ithaca have been made. The only difference so far observed is an apparently greater virulence both on tomato and potato of the California fungus.

So far as the North American continent is concerned, it seems that any specialized race of *P. infestans* on potato would be very widely distributed, particularly in view of the fact that the potato has been in cultivation on the continent for less than 300 years, that in many places its culture is not yet 50 years old, and especially in view of the extensive movement of planting stock from the extreme North to the extreme South and of table stock from the South northward, not to mention a certain inter-regional movement between East and West, especially in times of crop shortage in some region.

In the work here reported no attempt has been made to use a single strain of *P. infestans* for inoculation purposes. In fact, cultures or infected tissue have been secured from various places in New York, from West Virginia, and from Florida and used in making inoculations. It would have been easy to use pedigree cultures for tests made in the greenhouse. In the field, however, there is no way to prevent promiscuous infection, and there is every reason to believe that such infections have occurred at least in 1926 and in 1927. Nothing in the behavior of the potato cultures indicates that a new strain of the parasite has been introduced. Certainly the very small number of cultures that dropped in their recorded resistance can not be interpreted as an indication that this has happened.

If the question of specialization is considered from the intercontinental standpoint, it may be noted that Ekishirazu is equally as resistant in New York as in Japan. On the other hand a sample of Ekishirazu was sent to A. H. Cockayne in New Zealand and was pronounced (letter) susceptible by him. The only uncertainty about this evidence is that the tubers originally sent to Cockayne by the writer were relatively smooth-skinned, whereas Ekishirazu as grown at Ithaca usually has a rough skin. More typical samples were subsequently sent but no report on their behavior has been received yet.

Substantiating evidence for the occurrence of specialization may be seen in the fact that the seeds of Ekishirazu were obtained originally from some other country than Japan, possibly from France, but in any event a variety possessing the resistance of Ekishirazu was not known anywhere at the time those seeds were bought. This easily leads to the assumption that in some other places Ekishirazu would not prove resistant. So far as is known no test has been made.

In view of the well-known fact that blight very commonly appears first in Western Europe on the Atlantic coast and that it spreads rapidly eastward, it would seem that no biologic specialization is to be expected in Western Europe. The place to look for specialized forms would be far inland, as for example in Russia. Such evidence as exists for Russia, however, is (Bondartseva-Monteverde, 8, 9) that no specialization exists. If it should happen even occasionally that the spread of blight in Europe should be reversed this reasoning would be faulty. No evidence on this question has been found in the literature of the subject, voluminous as it is.

ORIGIN OF PHYTOPHTHORA INFESTANS

The origin of these resistant and semi-resistant plants is a most interesting subject for speculation. It was Goodrich's idea that he should go to the land where the potato is endemic to find plants with which to regenerate the common cultivated potato. This may have been an original idea with Goodrich but more likely, perhaps, was acceptance of the generally prevalent idea credited by Salaman (46) to Parmentier in 1786, that the potato had degenerated because of repeated asexual propagation. At any rate the thought antedated by several years the most gratifying demonstration (from the standpoint of plant pathology) of the soundness of the idea, namely, the success in combatting *Phylloxera* of the grape by the use of wild stocks from North America. For Goodrich, and those who denied the causal relation of *Phytophthora infestans* to "the disease," the reasoning is good, but for the modern pathologist no analogy exists. The French went to the land where the parasitic *Phylloxera* was endemic, and to make the

analogy perfect it must be assumed that *Phytophthora infestans* is also endemic to the higher Andes of South America. Upon looking for evidence that blight was known to the Indians or to the Conquerors it appears that practically none exists. Cieza (16) who mentions potato culture of the Indians does not mention any disease of the crop. Münter (36), as well as Vowinckle (51), cite a passage from Acosta (1) which is interpreted to mean that Acosta had observed potato rot in South America, presumably at Juli on Lake Titicaca where he spent most of his life. The word used by Acosta is "anublan." If a contemporaneous Spanish dictionary is consulted it will be seen that this verb might be used technically to indicate the production of injury by the burning of tissue by the sun's rays when passing through droplets of water. Acosta's whole "history" is free from technical terms, and it seems unlikely that he should have used this one word in a technical sense. Furthermore the context leads one to believe that he meant nothing more than that the potatoes sometimes "spoiled" in the ground because they became frozen. This is the meaning given by contemporaneous translators (Regnault, 1600 and 1616 and Grimston, 1604) of the Historia. The Acostan "evidence" proclaimed by Boussingault (10) refers to another Acosta entirely—an army colonel who was resident in Colombia at the time. It is extremely difficult to see how the colonel's testimony adds anything whatever to our knowledge of the disease. The only words in the letter, as published, that bear on the question are the following: "La maladie . . . est une espèce de champignon ou excrescence qui se développe sur différents points, et qui corrode plus ou moins profondément ces tubercules." Garcilasso de la Vega (18) makes it perfectly clear that the potato is "inclined to rot soon on account of its moistness." This statement is made in a chapter which treats of the storage depots for provisions and is part of a description of the freezing and drying process to which potatoes were subjected in order to make chuñu, a food material which can be stored indefinitely. This author had some knowledge of crop production in the Inca country from personal contact in the management of the vast estates of his father. He mentions potatoes in five different places in his Commentaries but never mentions disease. It must be inferred from this that the disease did not occur anywhere in the vicinity of Cuzco during several years at the middle of the sixteenth century. The Lagerheim (29) evidence (1890) is unmistakable but as this is of very recent date it is entirely possible that *Phytophthora infestans* was brought to Quito, or at least to South America, on European potatoes or other solanaceous hosts.

It seems a matter of some significance, too, that no record has been made of the occurrence of *Phytophthora* on specimens of South American Solanaceae collected prior to 1845. The natural inference is, considering that

every botanist in Europe was actively concerned with the disease and was serving on some national commission for investigating it, that all the sheets of tuberous Solani were critically examined for evidence of the disease and that if any lesions even remotely resembling blight had been found they would have been reported.

In viewing the matter from another angle, it will be recalled that deBary long ago pointed out that the potato might not be the natural host of *P. infestans*. He based this idea on the fact that the fungus does not form oospores on the potato, whereas practically all other members of the Peronosporeae do produce their oospores on the plants that they attack. DeBary searched for oospores on *Schizanthus grahami* without success.

The failure of *P. infestans* to reach Europe until nearly 300 years after the potato was introduced is likewise an important support to the idea that the fungus is not endemic in South America. The well-known explanation of Jensen (27, pp. 154–156) to account for this situation has been siezed upon eagerly and embraced by everyone who has need for some explanation of this circumstance. At the outset it may be assumed that potatoes were rarely brought to Europe except by accident. There was no demand for them and there is no likelihood that they would be carried as ships' stores except in cases where provisions had to be taken on in South America or from some captured vessel. When potatoes finally became an article of diet, however, active interest was aroused in the growth of the crop, and one of the most popular remedies proposed for the suppression of the widespread disease known as curl was to send to South America for new stock.

For many years, and in particular throughout the seventeenth century, every Spanish port of South America was closed to transoceanic traffic except Nombre de Dios on the north shore of the Isthmus. Prescott (40, Bk. 2, chapt. 3) cites manuscript sources to show that Pizarro himself, on his second attempt to reach what is now known as Peru, subsisted in part upon potatoes. A few years later Cieza (16) found potatoes under "cultivation" by the Indians at Popyan, Pasto, and many other places in Colombia. Any movement of these strange new "roots" from this region and the course that those tubers actually did take that were first introduced into the old world must have been down to the Pacific, north to Panama, across the Isthmus to Nombre de Dios and thence to Cadiz in Spain, unless perchance they were intercepted by buccaneers and carried elsewhere. Owing to the coldness of the Humboldt current these tubers need not have been subjected to a temperature exceeding 24° C. until they reached Panama. Here, and again at sea level on the Atlantic, the temperatures are higher. The data compiled by Reed (42) show a mean maximum temperature for Ancon (Pacific) up to 33.3° C. in March and one for Colon (Atlantic) not

exceeding 29° C. at any time in the year. The data also show that during the months of September, October, and November the mean maximum temperature at Ancon does not exceed 30.5° C. At Ancon the mean minimum temperature ranges from 23° C. downward and at Colon it ranges from 25° C. downward for the several months of the year.

Once out of port the hold temperatures of the small vessels then in use would approximate that of the surface water of the Caribbean. The climatological data of Reed (43) with additional data on surface water temperatures, kindly furnished by the Climatological Division of the United States Weather Bureau, show that at most times in the year this would be about 27° C. and that at no time would it exceed 29° C. In confirmation of this, an official of the United Fruit Company has supplied the information (letter) that the hold temperature of non-refrigerated vessels in Caribbean waters does not exceed 29.3° and rarely is more than 28.3° C. It appears from this that it would have been possible for months at a time to have moved potatoes from the higher Andes or the west coast of South America to European points without subjecting them at any time to a temperature high enough and prolonged enough to have killed any mycelium of *P. infestans* that might have been present. Jensen (27, p. 84) states that mycelium of *P. infestans* in tubers was killed in 65 hours at 30° C. but that it was living after 72 hours at 27.5° C. Jensen's data are by no means corroborated by the recent work of Vowinckle (51), who shows that mycelium grows in the parenchyma of the tuber at temperatures between 3.2° and 30.3° C. At continuous exposure to the latter temperature the mycelium had progressed 4 mm. in 13 days. This leaves a margin of more than a degree between the highest hold temperatures and the thermal death point of the fungus after an incubation of nearly two weeks. The effect of intermittent high temperatures has not been studied except that, in the field, mycelium in leaves and stems occasionally is subjected to temperatures of 32° C. or more for several hours at a time without being destroyed.

The presumption that "wild" potatoes from South America would prove resistant to blight seems to have been in the mind of practically every one who has given much thought to the control of the disease by biological means. The idea of degeneration through continued asexual propagation seems to have been back of the presumption. Goodrich had this idea, and Darwin seems to have had a similar thought. "Darwin's potato," *S. maglia* Molina, was employed in breeding by James Torbitt (Müller, 34) but was finally reported (17) susceptible. Heckel's (22) *S. commersonii* Dunal never has yielded anything except some "sports" about which there has been and still is much controversy. One of the

mutants, however, was reported by Heckel and Verne (23) to be resistant to blight. If the collection of material brought back from South America by André (2, 3) has yielded anything of Phytophthora-resisting value, it is not yet evident in the literature nor in the varieties recently listed by French seedsmen. Of the South American material obtained by Verne in 1911, one very encouraging report was made by Heckel and Verne (23) in which *Solanum acaule* Bitter, certain mutants, and the variety Luque amarga from the LaPaz market were recorded as immune. This report was based on the observations of a single year, and no further records seem to have appeared. Müller (34, p. 69), however, has two highly resistant races, one of which ("S.W.") is a hybrid of a South American plant grown by the Chilote Indians with the variety Switez, the other ("Ef") presumably of South American origin but received from a colleague in Berlin without label.

A few "wild" plants have been tested for blight resistance recently at Ithaca, both in greenhouse and in field, but all have been found susceptible. These include three samples of *S. commersonii* (one with a colored tuber, two with white) from the coastal plain of Southeastern South America; *S. maglia* from the island of Chiloé; *S. fendleri* Gray and *S. jamesii* Torrey from Southwestern United States; also a miscellaneous lot of "Indian potatoes" obtained on the market at Cuzco, and comprising perhaps a dozen different sorts.

It can not be denied that Goodrich obtained a resistant plant. His Rough Purple Chili was subjected to severe test through several serious epiphytotics. No stock of this variety is known to exist at present, although Goodrich offered it to the public and it was widely distributed. Its resistance, however, is extant in a number of varieties, and crops out from time to time when a particularly fortunate cross or selfing of these varieties occurs. It is unfortunate that the exact origin of this variety is unknown. Goodrich (20) states that the tubers came originally from Peru, but whether they did or not can never be determined, for it is not known how the original question of their origin was put to the saleswoman in the market at Panama where they were actually obtained. Three other lots of tubers of more certain South American origin were discarded by Goodrich because they were unsuitable for culture and also because they were somewhat susceptible.

The foregoing is simply the development of an idea originally suggested by W. F. Wight of the U. S. Department of Agriculture in personal correspondence. Mr. Wight's idea is that *Phytophthora infestans* is not endemic in South America; else the potato which unquestionably was carried to Europe from there, and not from Roanoke (Wight, 52), would not be so

universally susceptible. In a later letter Mr. Wight suggested that the fungus might have come originally from tomato. The suggestion has much in its favor. The occurrence of blight on tomato is decidedly rare. The only exceptions to this to be noted in North America are two small areas, one in California and one in the Piedmont region of the Alleghanies. When blight occurs in tomato elsewhere it is a matter of special comment. For the most part this seems to be the case in other continents.

At Ithaca, tomatoes of two or three different varieties have been grown very near the potato experimental field in each of three years without showing any infection whatever. A few volunteer tomato plants in the potato rows were inoculated regularly in 1926 but none ever showed infection. It is possible that foliage of this variety is immune to *Phytophthora*, as is that of certain varieties tested by Bondartseva-Monteverde (8). No fruits developed on these plants. Similar negative results have been obtained at times on tomato foliage in greenhouse tests. Whether these were indications of foliage immunity or whether the plants were not left long enough in the moist chamber after inoculation is not known for it was not known until the appearance of Berg's data that this factor was involved. Inoculated potato plants usually have not been held in the moist chamber for more than two hours, and Vowinckle's data as well as our own experience indicate that this is much longer than is necessary when fully matured swarm-spores are used for inoculation on foliage of susceptible varieties of potatoes.

The fact that some varieties of tomatoes show foliage immunity to *Phytophthora* is a substantial support to Wight's suggestion. It is practically certain that tomato breeders have given no thought whatever to Phytophthora rot or blight in their work so that the persistence of resistance indicates that this was characteristic of ancestral forms. Wight suggests (letter) that the original home of the tomato is still a matter for investigation. The rôle that the *S. melongenum* may play here has been implied earlier in this paper.

It is not worth while at present to carry speculation too far nor to do more than enumerate some of the difficulties involved. The ancestral type of *S. tuberosum* has not been established with certainty; the name *tuberosum* was clearly applied to a plant in heterozygous condition and unquestionably one subjected to a type of cultivation and even of selection for an unknown number of generations; many or perhaps most of the samples of "wild" *tuberosum* taken to Europe were not of the ancestral type at all but merely escapes; many of the specimens, especially in the earlier years, unquestionably came from irrigated regions where *Phytophthora* could not exist and where the natural selection process could not operate; tracing the

introduction of the commonest plants into new countries is often difficult or impossible—witness the case of the potato itself—and to trace the introduction of inconspicuous Solanaceae often presents even greater difficulties; whether to begin with boreal forms, as the biology of the fungus indicates, is even a question.

Obviously the difficulties are numerous; and clearly for the elucidation of these problems as well as for the confirmation of Vavilov's (49) paradox, the dominance of the recessives, more intensive study must be made of the Solani, especially of the boreal forms as they exist in their native habitat.

SUMMARY

The potato variety Ekishirazu from Japan has remained highly resistant to the attacks of *Phytophthora infestans* at Ithaca, New York, from 1921 to 1927 inclusive, but the variety has no commercial possibilities in New York. Hybrids with various varieties have yielded 46 families of plants possessing the approximate resistance of Ekishirazu, some of them decidedly tolerant to dry weather and some worthy of tests on a commercial scale.

Phytophthora infestans is regarded as exhibiting a low order of parasitism. It is not known to have more than one biologically specialized form.

Phytophthora infestans is not thought to be endemic to South America but to have been introduced there and into Europe on some other solanaceous host.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

LITERATURE CITED

1. ACOSTA, PADRE JOSEPH DE. Historia natural y moral de las Indias. Sevilla, 1590. The "fielmente reimpresa, Madrid, 1894" consulted. Book 1, Ch. 17.
2. ANDRÉ, ED. La patrie de la pomme de terre. Illustr. Hortic. **24**: 113–115. 1877.
3. ———. Sur l'origine et les variétés de la pomme de terre. Rev. Hort. **72**: 320–323. 1900.
4. APPEL, G. [!] O. Beitrag zur Frage der Immunitätszüchtung der Kartoffel. Pflanzenbau **4**: 51–56. 1927.
5. BARY, A. DE. Researches into the nature of the potato-fungus, *Phytophthora infestans*. Jour. Roy. Agr. Soc. England, II, **12**: 239–269. 1876. Reprinted in Jour. Bot. Brit. and Foreign **14**: 105–126, 149–154. 1876.
6. BERG, ANTHONY. Tomato late blight and its relation to late blight of potato. W. Va. Agr. Exp. Sta. Bul. 205. 1926.
7. [BERKELEY, M. J.] [*Botrytis infestans* in fruit of tomato.] Gard. Chron. and Agr. Gaz. **1848**: 52. 1848.
8. BONDARTSEVA-MONTEVERDE, V. N. *Phytophthora infestans* on tomato. In Russian. Bolezni Rastenii **15**: 1–27. 1926.
9. ———. A supplementary observation concerning *Phytophthora infestans* on tomato. In Russian. Bolezni Rastenii **16**: 76–81. 1927.

10. BOUSSINGAULT. Communique. Compt. Rend. Acad. Sci. Paris **21**: 1114–1115. 1845.
11. BROILI. Arbeiten mit Wildbastarden von Solanum. Mitt. Biol. Reichsanst. Land.- u. Forstw. **16**: 154–158. 1921.
12. BRUYN, HELENA L. G. DE. Waarnemingen over de vatbaarheid van het loof van de aardappelplant voor de aardappelziete. Tijdschr. Plantenziekten **32**: 1–29. 1926.
13. BRUYN, HELENA L. G. DE. The saprophytic life of *Phytophthora infestans* in the soil. Meded. Landbouwhoogesch. Wageningen **24**[1]: 1–37. 1922.
14. CANDOLLE, ALPH. DE. Sur une pomme de terre du Mexique. Rev. Hort., Ser. 4, **1**: 211–217. 1852.
15. ———. *Solanum tuberosum*. Géogr. Bot. Rais. **2**: 810–816. 1855.
16. CIEZA DE LEON, PEDRO DE. La Chronica del Peru. Seville, 1553. English translation by Clements R. Markham, London, 1864.
17. [Darwin, C.] [A report of the British Assoc. meetings] Gard. Chron. and Agr. Gaz. **1847**: 589. 1847. (The transactions do not contain any remarks by Darwin. One of the formal papers which was presented might easily have given occasion for the remark as quoted.)
18. GARCILASSO DE LA VEGA. Los comentarios reales de los Incas. Lisbon, 1609–1617. Edition of Horacio H. Urtega, Lima, 1918–20. English translation by Clements R. Markham, London, 1869–71.
19. GOODRICH, C. E. Potato disease. Trans. N. Y. St. Agr. Soc. **7**: 425–426. 1848.
20. GOODRICH, CHAUNCEY E. The potato; its diseases, with incidental remarks on its soil and culture. Trans. N. Y. St. Agr. Soc. **23**: 103–134. 1864.
21. HASKELL, R. J. *Phytophthora infestans* on eggplant in the United States. Phytopath. **11**: 504–505. 1921.
22. HECKEL, ÉDOUARD. Contribution à l'étude botanique de quelques Solanum tubérifères. Ann. Fac. Sci. Marseilles **8**: 101–115. 1898.
23. ———, ET CLAUDE VERNE. Nouvelles observations techniques sur les mutations gemmaires culturales des Solanum tubérifères (mutation de *S. tuberosum, jamesii* et *immite*). Bul. Soc. Nat. Agr. France **73**: 612–628. 1913.
24. ITO, SEIYA. A preliminary report on a late blight resistant strain of potato. Ann. Phytopath. Soc. Japan **1**: 5–8. 1918.
25. JEHLE, R. A. Disastrous effect of mosaic on the McCormick potato. Md. Agr. Exp. Sta. Bul. 282. 1926.
26. ———. Report on late blight of potato. U. S. Dept. Agr., Plant Disease Reporter **11**: 170. 1927.
27. JENSEN, J. L. Moyens de combattre et détruire le *Peronospora* de la pomme de terre. Mém. Soc. Agr. France **131**: 31–156. 1887.
28. JONES, L. R. The diseases of the potato. Trans. Mass. Hort. Soc. **1903**[1]: 144–156. 1903.
29. LAGERHEIM, GUSTAVO DE. La enfermedad de los pepinos. Su causa y su curacion. Revista Equatoriana **2**[24]. 1890.
30. LESLEY, J. W. Potato breeding in Great Britain. Rept. Potato Assoc. Amer. **8**: 52–58. 1922.
31. LINDLEY, JOHN. Notes on the wild potato. Jour. Hort. Soc. London **3**: 65–72. 1848.
32. LÖHNIS, MARIA PETRONELLA. Onderzoek over *Phytophthora infestans* (Mont.) deBy. op de aardappelplant. Proefschrift, Univ. Utrecht. 96 pp. Wageningen, 1922.

33. MELHUS, I. E. Germination and infection with the fungus of the late blight of potato (*Phytophthora infestans*). Wis. Agr. Exp. Sta. Res. Bul. 37. 1915.
34. MÜLLER, K. O. Neue Wege und Ziele in der Kartoffelzüchtung. Beitr. z. Pflanzenzücht. **8**: 45–72. 1925.
35. ————————. Untersuchungen zur Genetik der Kartoffel. Arb. Biol. Reichsanst. Land.- u. Forstw. **15**: 177–213. 1927.
36. MÜNTER, JULIUS. Die Krankheiten der Kartoffeln. . . . Berlin, 1846.
37. ————————. Allg. Gartenztg. **17**: 315. 1849. Review in Bot. Zeitung **8**: 170. Zeitung **8**: 170. 1850.
38. PAYEN. Végétation du *Botrytis infestans* à l'intérieur des fruits du *Solanum lycopersicum, erythrocarpum* (tomate). Compt. Rend. Acad. Sci. Paris **25**: 521–524. 1847. English translation in Gard. Chron. and Agric. Gaz. **1848**: 51–52.
39. PETHYBRIDGE, GEO. H. Potato diseases in Ireland. Jour. Dept. Agr. and Tech. Instr. Ireland **10**: 241–256. 1910.
40. PRESCOTT, WILLIAM H. History of the conquest of Peru. Vol. I. Boston, 1856.
41. REDDICK, DONALD. Ito's potato variety Ekishirazu in New York (Abstr.). Phytopath. **13**: 55–56. 1923.
42. REED, W. W. Climatological data for Central America. Month. Weather Rev. **51**: 133–141. 1923.
43. ————————. Climatological data for the West Indian islands. Month. Weather Rev. **54**: 133–160. 1926.
44. SALAMAN, RADCLIFFE N. The inheritance of colour and other characters in the potato. Jour. Genetics **1**: 7–46. 1910.
45. ————————. Potato varieties. 378 p. Cambridge, 1926.
46. ————————. Degeneration of the potato—an urgent problem. Jour. Nat. Inst. Agr. Bot. 3: 39–51. 1925.
47. STUART, WM. Potato breeding and selection. U. S. Dept. Agr. Bul. 195. 1915.
48. SUTTON, ARTHUR W. Potatoes. Jour. Roy. Hort. Soc. **19**: 387–430. 1896.
49. VAVILOV, N. I. Geographical regularities in the distribution of genes of cultivated plants. In Russian. Trudy Prikl. Bot. Gen. i Selek **17**[3]: 415–428. 1927.
50. VERNE, CLAUDE. Sur les *Solanum maglia* et *tuberosum* et sur les résultats d'expériences de mutations gemmaires culturales entreprises sur ces espèces sauvages. Compt. Rend. Acad. Sci. Paris **155**: 505–509. 1912.
51. VOWINCKLE, OTTO. Die Anfälligkeit deutscher Kartoffelsorten gegenüber *Phytophthora infestans* (Mont.) deBy., unter besonderer Berücksichtigung der Untersuchungsmethoden. Arb. Biol. Reichsanst. Land.- und Forstw. **14**: 588–641. 1926.
52. WIGHT, W. F. Origin, introduction, and primitive culture of the potato. Proc. Potato Assoc. Amer. 3: 35–52. 1916.

OLEANDER BACTERIOSIS IN CALIFORNIA[1]

CLAYTON O. SMITH

INTRODUCTION

For the past ten years or more a disease of *Nerium oleander* L. has been found sporadically in the nurseries of California. While the disease has at no time become serious, it has persisted and has occasionally been troublesome to control. The interest in this disease is scientific rather than economic and centers in its resemblances to and differences from other knot and gall diseases, such as olive knot (*Pseudomonas savastanoi* E. F. S.) and crown gall (*Ps. tumefaciens* Sm. and T.). It has been found occasionally in the open, on park and door-yard plantings. The slowness with which the disease has spread may be accounted for by several factors, chief of which are the strict quarantine and inspection of nursery stock for diseases, the unfavorable conditions in many places for the development of this disease, and the rather limited extent to which the oleander is used as an ornamental. At Riverside observations of the disease have extended over eight years. During this time it has persisted, varying in amount according to climatic conditions. The disease in 1926 was especially severe on the seed pods, peduncles, and young leaves. The rainy weather of April and the cool weather of May and June were apparently favorable for a heavy infection.

Considerable confusion has arisen regarding the nature of the oleander disease, largely through the inadequacy of the study thus far given it. It is with the hope of elucidating certain of these disputed points that this disease has been studied again. The results here given are based on data extending over a period of several years of intermittent study. The work has of necessity been largely a comparative study of the three organisms known to cause galls on trees. These three organisms, *Pseudomonas tumefaciens* from peach, *Ps. savastanoi* from olive, and the one from the oleander, have been grown and studied in pure cultures which have been used in making artificial inoculations.

HISTORY AND PREVIOUS STUDIES

Peglion (4) described a tubercle disease of oleander from Italy. He observed certain resemblances to the olive knot and isolated a schizomycete

[1] Paper No. 176, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

from the affected tissue. He made inoculations but published before securing results.

E. F. Smith (8) states: "The organism isolated by Peglion is unlike the olive-tubercle organism, and in Dr. Petri's experiments and in my own, a culture of it having been given me by Dr. Petri, also proved nonpathogenic to the oleander."

C. O. Smith (6) described a bacterial disease of oleanders in California. He cultivated a species of bacteria from the diseased lesions, studied its cultural characteristics in different media, and produced infection with it on the oleander and olive. No artificial inoculations at that time were made on the oleander with the olive-knot organism, then called *Bacillus olea*, but more recently described as *Pseudomonas savastanoi*.

E. F. Smith (8), in his study of the olive-knot organism, artificially inoculated oleanders with it, also making check inoculations on the olive. Negative results were secured with the inoculations on the oleander. He suggests that the oleander tubercle seems to him to be due to *Pseudomonas tumefaciens*. His observations and inoculation experiments (11) led him to think that the oleander disease is not due to the olive-tubercle organism. He also states that this was Petri's opinion.

Smith, Brown, and Townsend (12, p. 31 and pl. 3) also describe and illustrate successful inoculations of the oleander with *Ps. tumefaciens*, and for comparison picture an oleander, having a natural gall, from California. They note that in some instances these artificial galls produced by *Ps. tumefaciens* are very similar in their youngest stages to the young natural galls on oleander received from California. In a footnote (12, p. 32), they state that the organism causing the California disease of oleander is probably not identical with *Ps. tumefaciens*. Artificial inoculations of the olive with *Ps. tumefaciens* gave negative results (12, p. 33, 34, 88).

Savastano (5) concluded a series of artificial inoculations with pure cultures of the olive organism, as well as fragments of olive tubercles, into healthy oleander trees. He states that the bacterial diseases of the olive cannot be transmitted to the oleander.

Evans (2) reports the oleander disease as being of frequent occurrence both in the Transvaal and in Cape Colony. Alcoholic-preserved material of the oleander bacteriosis sent by Evans seemed to be typical of the oleander trouble as found in California, and a microscopic examination of stained sections showed pockets of bacteria in the tissue.

Tonelli (15) describes in some detail the bacteriosis of the oleander in Italy. The disease was found by him in gardens and parks. He isolated a schizomycete from the tumors, made successful inoculations on oleander, and made a limited study of the cultural characteristics. He noted that

negative results were secured when *Chrysanthemum frutescens* was inoculated with the oleander organism, even after five months' time, while positive results were secured with *Ps. tumefaciens* in chrysanthemum and oleander.

Hariot (3) reports the oleander disease in France and refers to the work and views of Tonelli.

The oleander bacteriosis has been reported from Italy, France, South Africa, and California. It probably had its origin in Italy, and was distributed on nursery stock to the other subtropical countries.

SYMPTOMS OF THE DISEASE

The infection on the oleander has been found as aerial galls or lesions on the stems, foliage, and inflorescence (on the peduncles and the ovary, and in artificial inoculations on the petals). It has not been found on the root or on any underground part of the stem, in this respect differing from infection produced by the crown gall organism, *Ps. tumefaciens,* which normally develops on the roots and the underground portion of the trunk. There is considerable variation in the form of the oleander lesions or knots, depending upon the nature of the tissue infected and possibly upon the season of the year in which infection has taken place.

On the older stem tissue the organism develops a more or less spherical knot or gall (Pl. VII, C–D) that may project 1–2 cm. and is 1–2 cm. in diameter. On the less lignified twig the organism forms more of a canker, the hypertrophy taking place within the tissue immediately surrounding the injury or puncture, where it may cause the healthy stem tissue to split longitudinally (Pl. VIII, D).

The knots consist of rather soft or spongy tissue; they are rough, irregular, and at length blackish in color. If the tumor tissue is cut away, small dark-colored areas or pockets (Pl. VIII, F–G) can be seen in the tissue, which show numerous bacteria. These spots are usually found next to the healthy tissue. The natural knots or infections are often found localized in the leaves (Pl. VII, A) or at the nodes of the stem (Pl. VII, C), in this respect agreeing with the olive knot, *Ps. savastanoi.*

On more succulent tissue, the disease is not always localized in a knot, but develops a definite lesion which may spread upward and less often downward in the tissue from the initial inoculation (Pl. VIII, H–J). In this case there is often a distinct channel of infection, recognized externally by a longitudinal swelling of the tissue and often by the appearance of small roundish swellings or secondary smooth tubercles (Pl. VIII, H; Pl. IX, A). The infection on the stem may cause hypertrophy along one side of the branch, thus causing more or less torsion. This hypertrophy often

develops at the extremity of the branch as an enlarged mass of chlorotic tissue or a gall-like canker.

The foliage is variously infected. The disease may be localized at a single point, causing a conical elevation of the tissue, usually toward the upper surface of the leaf (Pl. VIII, A). It may cause a gall very similar to those on the stem but of smaller dimensions (Pl. VII, Ae). Surrounding this hypertrophy is sometimes a yellowing of the tissue as shown in Plate VII, Ab. In an infected leaf, hypertrophies or small secondary tumors are often found on the midrib and on the veins branching from it (Pl. VII, B). The secondary infection in the leaf is carried in the circulatory system of the leaves, possibly in the laticiferous elements that occur in the veins. Young leaves are often infected almost as soon as they unfold from the bud, and show various forms of torsion and curling, depending upon the side on which the hypertrophy has developed. They are often characterized by a marked thickening of leaf tissue and inrolled margins (Pl. VII, A). The midrib often bends toward the stem. When the smaller veins are infected the surface of the leaf is bent toward the upper or lower side, depending upon where the hypertrophy has taken place.

The seed pods become infected in park—and outdoor plantings when the disease is prevalent. This suggests that the organism may be distributed by insects, possibly by bees.

The pistils[2] are infected in various stages of development, and are diversely distorted (Pl. VII, E–G). If the seed pods have reached some size when infected, they become curved and distorted but produce normal seed. If they are attacked early, as in flower, they become more or less bottle-shaped, are shorter and thicker than normal, and have aborted seeds. The dead petals (Pl. VII, C) remain attached to the infected pistils for a long time after blooming.

CHANNELS OF INFECTION

No tumor strands similar to those which Smith (10, p. 81) has found in his study of crown gall on tobacco have been observed in the study of the oleander knot, but definite channels of infection (Pl. IX, A, E) often develop in the active growing tissue of naturally and artificially inoculated stems and leaves. These infection channels may number from one to several in severely infected tissue (Pl. VIII, H–I). At times the infection from artificial inoculations may become general, and be distributed more uniformly throughout the infected tissue, but even here more or less definite

[2] The author wishes to acknowledge the courtesy of Dr. Antonio Tonelli, Valsalice (Torino), Italy, for specimens of the European oleander disease, and also for his observation on the infection of seed pods.

lines of infection are usually present, as indicated by the secondary tumefactions.

The channels of infection may originate from a primary inoculation and be arranged in nearly straight lines that often extend through several internodes (Pl. IX, A; Pl. VIII, H–J).

In their early stages of development they are swollen and have a more transparent consistency and a lighter green color than normal tissue. From the channels of infection secondary tumors are developed as small, smooth swellings of the tissue. When their surface is cut away a dark-colored pocket is found which can be traced to a dark-colored strand of tissue usually situated in the cortex of the stem (Pl. VIII, F, G, K). This condition closely resembles the secondary tumors of the olive knot as described by Smith (9, p. 71, 72, and 192; 11, p. 387), who says, "A distinct channel of infection is traceable from the secondary tubercle." These infection channels are found in the cortex of the oleander and are apparently distinct from the vascular system, but may possibly in their earlier stages be associated with the laticiferous vessels.

Ps. tumefaciens, when inoculated into oleander, has a tendency to form secondary tumors (Pl. IX, J) which are situated near the primary inoculation. No typical tumor strands (metastases) have been observed in the inoculation of oleander with *Ps. tumefaciens*.

In the leaves the channels of infection apparently follow the veins but are apparently distinct from the vascular system, as cross-sections of veins and petioles fail to show the organism present in the vessels, while masses of bacteria are readily found in the parenchyma. It is believed, but not with certainty demonstrated, that the organism may use the laticiferous vessels which are found in the veins.

The oleander knot often spreads by infection channels from the stem to the leaf tissue and vice versa. It may spread from an inoculation in the midrib to the secondary veins (Pl. VII, B).

ARTIFICIAL INOCULATIONS

The oleander bacteriosis organism has been successfully inoculated into oleander, *Nerium oleander* (Pl. VIII, D–K), the olive, *Olea europea* Linn, (Pl. VIII, C), and certain other hosts related to the olive, as *Adelia* (*Forestiera*) *acuminata* Michx. (Pl. VIII, B) and *Chionanthus virginica* L. (Pl. VIII, A). Negative results were secured with the oleander organism on *Fraxinus floribunda* (S. P. I. 47687, United States Department of Agriculture, Bureau of Seed and Plant Introduction), *F. velutina* Torr., *Osmanthus ilicifolia* Lieb., *Osmanthus fragrans* Lour., *Schinus molle* L., *Prunus salicina* Lindl. × *P. simonii* (Wickson plum), *P. cerasifera* Ehrh., *P. simonii*

Carr., *P. eriogyna* Mason (synonymous with *P. fremonti* S. Wats.), *Amygdalus persica* L., *A. communis* L., *Vinca*, *Coprosma baueri* Endl., *Thevetia nereifolia* Juss., *Carissa grandiflora* DC., and *Chrysanthemum frutescens* L. The above species of *Prunus* are especially susceptible to the crown-gall organism.

On *Thevetia nereifolia*, the area about the puncture was dark in color and contained a limited amount of dead tissue, but no typical lesions developed. Small hypertrophies are believed to have been produced (only once) on a species of privet, these being little more than knobs on the margin of the healing tissue.

The oleander bacteriosis is readily reproduced artificially in oleander by puncture inoculations as recorded by C. O. Smith (6) and Tonelli (15). Repeated successful artificial infections on both olive and oleander have been made from pure cultures or with diseased oleander tissue. The artificially produced hypertrophies on the olive (Pl. VIII, C), however, differ from those on the oleander (Pl. VIII, E) in being more nearly spherical, or sometimes irregular, elevated growths which enlarge rather rapidly into simple or multiple gall-like tumors consisting of soft tissue extending beyond the stem for 2 to 3 or more centimeters. This abnormal tissue remains green for several months with but little necrosis. During the second year little new development of the knots takes place, and at the end of the season the tissue of the twig is dry and dead as far back as the normal healthy tissue. It is thought that these galls are much shorter-lived than the true olive knot caused by *Pseudomonas savastanoi*. No metastasis, secondary tumors, or channels of infection have been observed in the artificial inoculations of the olive with the oleander organism.

These artificial galls (Pl. VIII, C) on olive produced by the oleander organism have certain resemblances to the olive knot (Pl. VII, H) and to the galls produced on other hosts by *Pseudomonas tumefaciens*, although apparently differing somewhat from both. The olive knot due to *Ps. savastanoi* is usually more regular in shape and has a smoother surface and a wider base of attachment than the oleander knot on olive. *Ps. tumefaciens* apparently does not infect the olive. This statement is based on the negative results from large numbers of artificial inoculations with a strain of *Ps. tumefaciens* isolated from *Prunus* (peach, almond, and plum). The virulence of this strain of *Ps. tumefaciens* was tested by other positive inoculations made at the same time and place on different kinds of *Prunus*. Smith, Brown, and Townsend (12) record several unsuccessful attempts to inoculate the olive with *Ps. tumefaciens* isolated from Paris daisy, peach, and hop. In an investigation by Smith and Quirk (14, p. 505) it is shown that young olive shoots have a highly acid juice (pH 5.20 to 5.28), while the acid

limit of *Ps. tumefaciens* is only about pH 5.70 in peptone beef bouillon. The acidity of the olive is probably the chief factor making it resistant to *Ps. tumefaciens*. It will be noted that *Ps. savastanoi* and the oleander organism have a somewhat lower pH limit (about pH 5.3 in peptone beef bouillon) than *Ps. tumefaciens*.

The oleander organism artificially inoculated into the oleander usually causes slight hypertrophies to develop around the puncture and within the wounded tissue, often causing it to split apart by the abnormal growth. In young, rapidly growing tissue the infection causes the tissue to become swollen and to take on a clear, lighter green color, often with a water soaked appearance near the outer edge of the margin.

A darkening of the surface and the interior tissue surrounding the lesion soon takes place, and is to be found beneath both the secondary infections and the primary lesions, often extending in the latter case into the pith. Within this abnormal tissue and near the healthy tissue may often be found dark-colored pockets that contain numerous bacteria (Pl. VIII, F, G). The lesions that are externally evident are often rather large in size, but when inoculated tissue that to external appearance is apparently normal is cut into it may show blackened necrotic regions. The inoculation of oleander with *Ps. tumefaciens* always produces small galls (Pl. IX, J) rather than lesions or cankers.

The young pistils of the open flowers are readily infected by atomizing with a suspension of the oleander organism (Pl. VII, F). A few typical hypertrophies have also appeared on the inoculated petals.

The inoculations on *Chionanthus virginica*, white fringe, produce definite but usually not typical knots. They show around the punctures at first as an abnormal swelling, 2 to 3 millimeters in diameter, which is of a darker color and has a more or less water-soaked appearance. Some further inoculations made on new growth of *Chionanthus virginica* gave spherical knots (Pl. VIII, A) which were more like olive knot and somewhat similar to those produced by the oleander organism on olive. Sometimes cylindrical, point-like outgrowths develop on the same twig with the more spherical knots. In the inoculations of June 15 (see table 1) peculiar abnormal cylindrical growths appeared both on the stems (Pl. VIII, Ae) and on the leaf petioles (Pl. VIII, Ad).

The inoculations on *Adelia* gave spherical galls that were somewhat similar to those made by the oleander organism on olive. Dilution cultures from these knots as well as those of *Chionanthus* produced numerous colonies in petri plates. When transfers from these were inoculated into oleander, typical lesions and knots developed.

TABLE 1.—*The results of inoculating four different hosts with the oleander disease organism.*

Host inoculated	Date of inoculation, 1920	No. punctures made at inoculation	Knots produced	
			No.	Diameter (in mm.)
Chionanthus virginica	Apr. 4	10	1	5
	Apr. 10	10	7	5
	June 12	10	9	3
	June 15	20	6	6
Olea europea	Apr. 4	10	3	3
	Apr. 30	10	4	5
	June 12	10	5	12
	June 15	10	5	5
	June 23	20	14	5
Adelia acuminata	Apr. 30	10	6	3
	June 12	10	4	6
Nerium oleander	Apr. 4	10	0	—
	Apr. 30	10	6	3
	June 12	10	7	6
	June 15	10	5	5

PATHOLOGICAL HISTOLOGY

The presence of bacteria in the hypertrophic and hyperplastic tissue which had been produced by inoculation with the oleander organism was readily demonstrated by employing the usual histological methods. The tissue was killed in Carnoy solution and sectioned with a sliding microtome, or, where suitable, with a rotary one, from material embedded in paraffin. Acid fuchsin was used as the stain.

Infected tissue of *Nerium oleander* (oleander), *Olea europea* (olive), *Chionanthus virginica* (white fringe), and *Adelia* (*Forestiera*) *acuminata* was sectioned and studied. In these studies, tissue masses of different sizes were selected that had been artificially infected for various lengths of time. In the infected tissue when sectioned, a dark-colored area usually marked the location of the infected areas and channel of infection. This was especially true of the oleander, where these areas were found in the cortex, pith, or leaves.

A study of oleander tissue, showing infection channels and secondary tumors, was made from young, vigorously growing tissue, inoculated June 17 and fixed Oct. 27 (Pl. IX, E). A cross section of the affected area showed, in the cortex, small dark-colored circular or irregular areas, 1 milli-

meter or less in diameter (Pl. IX, F). A microscopic examination showed definite bacterial pockets. In the center were broken-down tissue and masses of the causal organism (Pl. IX, G). Surrounding the bacterial masses and disorganized tissue were several layers of small, thin-walled cuboidal cells. Around these were layers of larger, more or less flattened cells, which in turn were surrounded by normal cortical tissue.

Very young, rapidly growing tissue of oleander may develop a visible infection strand in from nine to forty days. Such material inoculated November 5 and fixed December 9 was imbedded in paraffin and sectioned (Pl. IX, Ae). Here a definite infection strand is shown extending between two primary inoculations, and through two internodes and then into the petiole of the leaf. Definite lesions developed at the points of inoculation but no typical galls.

In cross sections the tissue was seen to be enlarged on the side where the infection strand was visible. Five areas in the broken-down cortical tissue showed masses of bacteria indicated by x in Plate IX, B. At these points an increase in number of cells, but not in size resulted, and only parenchymatous tissue was formed (Pl. IX, C). The vessels adjacent were rarely found to be infected.

In another series of inoculations on oleander at the same time and place with a different culture, a similar infection strand developed in nine days. Longitudinal sections showed bacteria in masses occupying definite rows in the tissue. Two or more of these narrow bacterial masses may be parallel to each other as in Plate IX, D, where the organism may possibly have been distributed through the laticiferous vessels. This, however, requires further demonstration.

An inoculation of the midrib of an oleander leaf will often result in the infection developing through the entire vascular system of the leaf (Pl. VII, B). The bacterial pockets seem to have no definite location in the midrib. Sometimes a large mass of bacteria is at the center, while in other sections several smaller masses are situated in the parenchyma near the outside of the midrib. Similar masses of bacteria were found in the parenchyma of an infected petiole of oleander.

Sections of galls produced on the olive and on *Adelia* (*Forestiera*) *acuminata* by the oleander organism also showed masses of bacteria, often associated with necrosis of tissue. These pockets were sometimes located in the center of the gall and at other times in other parts of the enlarged tissue. The hypertrophies were for the most part parenchymatous, with vascular elements arranged in an irregular way. In a gall on olive, 15 millimeters in diameter, caused by the oleander organism, three dark-colored lines were found extending through nearly to its base, where they terminated in a

dark-colored center, an area in which numerous bacteria were found in mass (Pl. IX, I).

The infection on stems of *Chionanthus virginica* may develop into a peculiar branching growth 6 to 7 millimeters in length (Pl. VII, Ae). At each of the tips of this growth was a groove-like depression that seemed to divide in into two symmetrical parts. A cross section of these galls showed an interesting arrangement of tissue (Pl. IX, H). On the outside was a normal layer of bark epidermis, and within this was a layer several cells wide consisting of large and thin walled cells, that did not stain deeply with acid fuchsin. Next within this tissue was an area of smaller cells also not staining deeply. In the center of the latter tissue were two cavities evidently corresponding to the two branchings of the outgrowth. These cavities contained large masses of bacteria, which could be observed at the margin of the cavity where they had not been washed out in treatment. There was apparently no necrosis of the tissue in these knots. Some vascular tissue (spiral vessels) was found immediately surrounding the center cavity. Binucleated cells were occasionally found in the outer zone of this tissue. Galls similar to those on the stems were produced on the petioles of leaves of this host (Pl. VIII, Ad). The tissue showed an arrangement similar to that just described but with more vascular tissue present.

Sections of tissue infected with the oleander organism rarely failed to show numerous bacteria associated with broken-down tissue. This is in marked contrast to the absence of organisms as observed by Smith, Brown, and McCulloch (13) in their study of the structure of galls on hosts infected artificially by *Ps. tumefaciens*.

RELATIONSHIP OF CAUSAL ORGANISM

The artificial inoculations and the etiological and cultural characteristics indicate that the oleander organism is more closely related to *P. savastanoi* than to *P. tumefaciens*. Repeated inoculations with *P. savastanoi* on *Nerium oleander* have always given negative results, thus agreeing with the work of Smith (8) and Savastano (5), but the olive-knot organism, according to C. O. Smith (7), will infect certain hosts related to the olive, as *Adelia* (*Foresteria*) *acuminata*, *Chinanthus virginica*, *Osmanthus aquifolia*, *Jasminum primulinum*, *Fraxinus velutina*, and *F. floribunda*. The positive results of inoculation of the oleander organism on olive *Olea europea*, *Chionanthus virginica*, and *Adelia* (*Forestiera*) *acuminata*, and the negative results on species of *Prunus*, sugar beet, *Beta vulgaris*, *Thevetia*, and *Carissa* are significant in pointing to its relationship to *P. savastanoi*.

The careful work done by Smith, Brown, and Townsend (12) in studying various strains of *P. tumefaciens* has shown variations in cultural char-

acteristics of these organisms that are difficult to explain unless they are regarded as different strains or races or perhaps species.

The oleander and olive organisms have characteristics not differing so very much from certain of these strains, but they could hardly be regarded as belonging to the tumefaciens group. If the oleander organism is described as a new species, the following facts should be considered: (*1*) The olive organism *Ps. savastanoi* is not parasitic on the oleander, and cannot be made to cause knot on it; (*2*) the knots produced by the oleander organism on the olive differ somewhat from olive knots; (*3*) the cultural and morphological characteristics of the two are not sufficiently different to separate the two into different species; (*4*) the oleander strain often develops infection channels in the oleander which are very similar to those formed by the olive organism in the olive; (*5*) the two are not distinguishable morphologically. While the resemblances between the olive and oleander organisms are close and the differences difficult to detect, still these organisms are probably different strains although not distinct species. E. F. Smith (11, p. 404) believes that there are very likely several strains of *Ps. savastanoi,* all pathogenic but somewhat different. He describes (11) an ash disease from Europe that is very similar morphologically and culturally to the olive tubercle, and yet not absolutely identical. It is caused by an organism pathogenic on the ash, *Fraxinus excelsior,* but not on the olive, and this organism is regarded as a variety of *Ps. savastanoi* rather than as a different species. It is of interest to note that C. O. Smith (7) produced knots on *Fraxinus* artificially with *Ps. savastanoi* but not with the oleander organism, yet the latter did make knots on other hosts in addition to the olive and oleander. It seems best with our present knowledge to regard the oleander organism as being a variety or strain of *Ps. savastanoi* rather than to make a new species. The name *Ps. savastanoi* var. *nerii* is suggested.

Cultural Characteristics

Pseudomonas savastanoi var. **nerii** n. var.

Rods with rounded end, 1.5–2.5 × 0.5–0.6 μ; spores and capsules absent, flagellae polar, 1–3; colonies after 8 days on potato dextrose agar at 25° C., 1–3 millimeters in diameter, flat, circular, shining, often with a somewhat undulate margin. Young colonies with a bluish cast by transmitted light, carbon gray[3] by transmitted light, reticulations sometimes present especially near margin, which is of a paler (nearly hyaline) color, flaky white particles sometimes present, but transitory. Colonies after 8 days on gelatin smaller, 1–2 millimeters in diameter. Gelatin not liquefied or stained. Ex-

[3] Robert Ridgway. Color standards and color nomenclature, Washington, D. C., 1912.

cellent growth in nutrient beef agar; better growth in potato dextrose agar, spread out in a rather thin layer, often with an irregular lobed margin, shining, opalescent, changing to pale gray in 10 days. On litmus peptone dextrose agar slants, the litmus promptly (48 hours) and permanently changes to a bright red. Growth at first vigorous, but limited in amount and soon ceases. The same reaction takes place in litmus peptone galactose agar. In litmus peptone saccharose agar some purpling of medium near growth, deep vinaceous by reflected light.

In liquid medium, nutrient beef bouillon, showed dense cloudiness with a partial pellicle and small amounts of sediment. In beef bouillon under chloroform, unrestrained growth. In 2 per cent peptone no pellicle or precipitate formed, color becoming almost a honey yellow (100 days). Slight cloudiness in 2 per cent saccharose without pellicle or precipitate (35 days). In tubes autoclaved 15 minutes at 15 pounds pressure, slight cloudiness without ring or pellicle after 11 days, but densely clouded after 20 days. In maltose peptone cloudiness developed without pellicle or precipitate (10 days), becoming somewhat browned (100 days). Saccharose 2 per cent, peptone 1 per cent, produced a partial pellicle and ring formation, moderately clouded, somewhat browned in color.

No gas was produced in fermentation tubes (20 days) containing 2 per cent peptone and 1 per cent of the following carbon compounds: dextrose, galactose, lactose, saccharose, maltose, mannite, and glycerin. The liquid clouded promptly (5 days) and was confined to open arm. Litmus milk gradually blued, strong alkaline reaction (5–10 days). No whey or separation of casein, reduction of litmus slow, tendency for it to clear (2–3 months). Cultures continue to darken after inoculation; one series changed from lavender to pale vinaceous and pale Valey's gray (10 days), plumbago blue (25 days), slate and slate purple (90 days). The liquid at this time is one half of former volume but shows no whey. Methylene blue shows some fading of color (3 days); pale methylene blue (7 days), becoming tea green (14 days), and sage green and American green (4 months). Milk gave no separation of casein; white color gradually disappears, becoming ochraceous buff (15 days), pale ecru Dauthenay (1, p. 166, No. 4) (28 days); buff Dauthenay (1, p. 209) and in some tubes dark brown Dauthenay (1, p. 304) (4 months). At this time tubes had the appearance of clearing, but were not entirely so even after 6 months. Cohn solution gave cloudiness sometimes with and at other times without crystals. When transferred to Cohn solution from tubes of bouillon, the organism sometimes failed to grow; while good growth resulted when an agar slant was used. No growth on Uschinsky's solution. A positive indol test in 1 per cent peptone (Wittes) and distilled water, using the sodium nitrite

test. Standing or heating in hot-water bath was necessary to produce the pink color. Thermal death point is 51° C.

The pH limits of growth in peptone meat extract bouillon for *Ps. savastanoi* and *Ps. savastanoi* var. *nerii* are the same, being pH 5.3 to pH 8.9 (Colorimetric method).

SUMMARY AND GENERAL DISCUSSION

1. The oleander organism was grown on the same culture media as that of the olive knot, *Pseudomonas savastanoi*, and showed practically identical cultural characteristics. The differences are very slight and are pointed out in the descriptions of the oleander organism.

2. *Pseudomonas tumefaciens* (isolated from peach) differs markedly from the olive and oleander organisms in cultural characteristics, pathogenicity, and reaction on hosts. It is more vigorous in growth than those organisms and is apparently distinct from them.

3. In liquid media the olive and oleander organisms develop a fine cloudiness held in suspension and at times a partial or very thin complete pellicle, but sometimes only a ring-like formation. The following liquid media were used in its growth: beef bouillon, 2 per cent peptone solution, 2 per cent saccharose, 2 per cent dextrose, dextrose peptone solution, saccharose peptone, and maltose peptone solutions, and asparagin in solution.

4. *Pseudomonas tumefaciens* grew on the above liquid media in a very different manner, producing almost no cloudiness, but a growth of floccules and threads which formed a definite precipitate, or remained in suspension in the nearly clear liquid. A pellicle was almost always present and was often dense. Growth occurred in saccharose and saccharose peptone solutions, with inversion of this sugar.

5. The reaction of the olive and oleander in milk and litmus milk was very different from that of *Pseudomonas tumefaciens* (peach strain). The latter separated the casein after several days and reduced the litmus, at length developing a brown-colored liquid coagulum, while the other two organisms developed shades of blue and were always without a coagulum or whey. It is of interest to note that according to Smith, Brown, and Townsend (12, p. 154) certain strains of *Ps. tumefaciens* react in litmus milk very similarly to the oleander and olive organisms.

6. The reactions and growth on solid agars differ. *Pseudomonas tumefaciens* grows along the stroke in a piled-up manner. The other two have a flatter, more spread-out, less watery-appearing growth. The growth of *Ps. tumefaciens* on dextrose and galactose peptone litmus agars does not redden the litmus like the oleander and olive organisms, and its growth is unrestrained in this medium.

7. The oleander organism produces more or less evident galls on some hosts, while on others lesions are formed with hypertrophies within or at the margin of the healing tissue. The organism is found in masses in spaces occupied by broken-down tissue but not within the cell.

8. The oleander organism was pathogenic on the olive and certain hosts that are botanically related to the olive. It was pathogenic on the oleander (its natural host), but not on certain hosts that are related to the oleander. Some of the same hosts susceptible to the oleander organism were also susceptible to the olive knot organism when artificially inoculated. The oleander organism was pathogenic on olive (*Olea europea*), *Chionanthus*, *Adelia*, and *Nerium oleander*. The olive knot organism was pathogenic on the same hosts, with the exception of oleander and the addition of species of *Fraxinus* and *Osmanthus*. Negative results were secured with the olive and oleander organisms on species of *Prunus*, on *Chrysanthemum frutescens*, *Carissa grandiflora*, *Vinca* and *Thevetia nereifolia*.

9. The olive and oleander galls produced by the oleander organism showed definite pockets or masses of the causal organism. These in the oleander were readily detected with the unaided eye as small black areas in the greener, hypertrophied tissue. The same necrotic tissue was found when secondary tumors and infection strands were sectioned.

10. The characteristics of the oleander organism were not sufficiently distinct from those of the olive-knot organism to justify making it a distinct species. The pathogenicity was very similar except that the olive organism did not infect the oleander. For the present it seems best to regard the oleander organism as a variety of the olive-knot organism. If a name is desirable, *nerii* is suggested (*Pseudomonas savastanoi* variety *nerii* n. var.).

UNIVERSITY OF CALIFORNIA,
GRADUATE SCHOOL OF TROPICAL AGRICULTURE
AND CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. DAUTHENAY, HENRI. Repert de couluers, Soc. Fr. des Chrysanth. 365 col. pp.; Impr. Oberthur, Rennes. 1905.
2. EVANS, J. POLE. Knot disease (oleander) *Bacillus oleae*. Transvaal Ann. Rep. Dept. Agr. **1907–1908**: 126. 1909.
3. HARIOT, P. Canker of oleander. Bul. Soc. Path. Veg. France **2**: 28–40. 1915.
4. PEGLION, VICTOR. La rogna o tuberculosi del *Nerium oleander*. Rendic. Accad. Lincei. **14**: 462–463. 1905.
5. SAVASTANO, L. Sulla trasmissibilità del bacillo della tubercolosi dell'olivo nell'oleandro. Bol. Arbor. Ital. **4**: 86–87. 1908.

6. SMITH, C. O. A bacterial disease of the oleander. Bot. Gaz. **42**: 301–310. 1906.
7. ————————. Pathogenicity of the olive knot organism on hosts related to the olive. Phytopath. **12**: 271–278. 1922.
8. SMITH, E. F. Recent studies of the olive-tubercle organism. U. S. Dept. Agr., Bur. Pl. Indus. Bul. 131: 25–43. 1908.
9. ————————. Bacteria in relation to plant diseases. Vol. II. 368 pp. Carnegie Institute, Washington, D. C. 1911.
10. ————————. Crown gall studies showing changes in plant structure due to changed stimulus. Jour. Agr. Res. **6**: 179–182. 1916.
11. ————————. An introduction to bacterial diseases of plants. 391 pp. W. B. Saunders Company, Philadelphia and London. 1920.
12. ————————, NELLIE A. BROWN, and C. O. TOWNSEND. Crown gall of plants; its cause and remedy. U. S. Dept. Agr., Bur. Pl. Indus. Bul. 213. 1911.
13. ————————, NELLIE A. BROWN, and LUCIA MCCULLOCH. The structure and development of crown gall. U. S. Dept. Agr., Bur. Pl. Indus. Bul. 255. 1912.
14. ————————, and AGNES J. QUIRK. A begonia immune to crown gall; with observations on other immune or semi-immune plants. Phytopath. **16**: 491–508. 1926.
15. TONELLI, ANTONIO. Una bacteriosi del leando (rogua, o canero, o tubercolosi de leandro). Ancoli R. Accad. della Agricolt. di Torino **55**: 383–400. 1912.

EXPLANATION OF PLATES

PLATE VII

Oleander tissue except in H. A: Natural infection on leaves; light areas indicate yellowing of tissue. Observe rolling of young leaves and conical infection on margins. B: Artificial inoculation in midvein and subsequent invasion of vascular system. C and D: Oleander stem from a park planting. Note at upper left the infected pistils with petals still attached. E: Diseased oleander seed pods sent from Italy by Dr. Antonio Tonelli. G: California infected oleander seed pods.

H. Natural knots on olive caused by *Ps. savastanoi* for comparison with those on olive by oleander organism (Plate VIII, C).

PLATE VIII

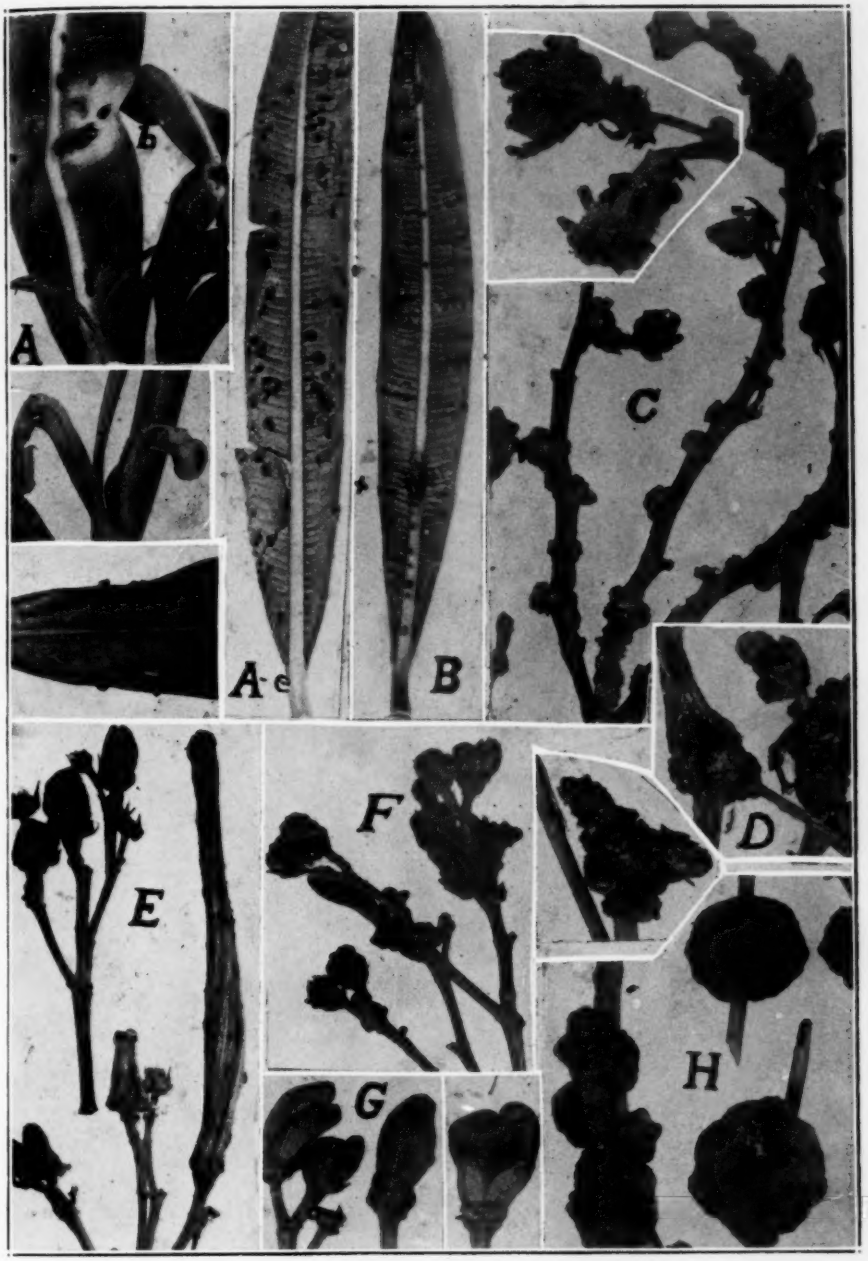
Artificial inoculations with oleander organism. A: Knots formed on white fringe (*Chionanthus virginica*) after three months. Peculiar outgrowths are shown on leaf petiole and on two twigs at right. For cross section of e see plate IX, H. B: Knots on *Adelia* (*Forestiera*) *acuminata* after three months. C: Knots on olive after five months. D: Splitting of oleander tissue by growth within. Photographed six months after inoculation. E: Knots on oleander after 35 days. F and G: Surface of J cut away revealing the darkened interior tissue and bacterial pockets. H: Secondary lesions formed on oleander between primary inoculations at **x**. I: Elongated secondary tumefactions on oleander. J: Channel of infection on oleander that developed from positive artificial lesions at **x**. K: Infected cortical oleander tissue.

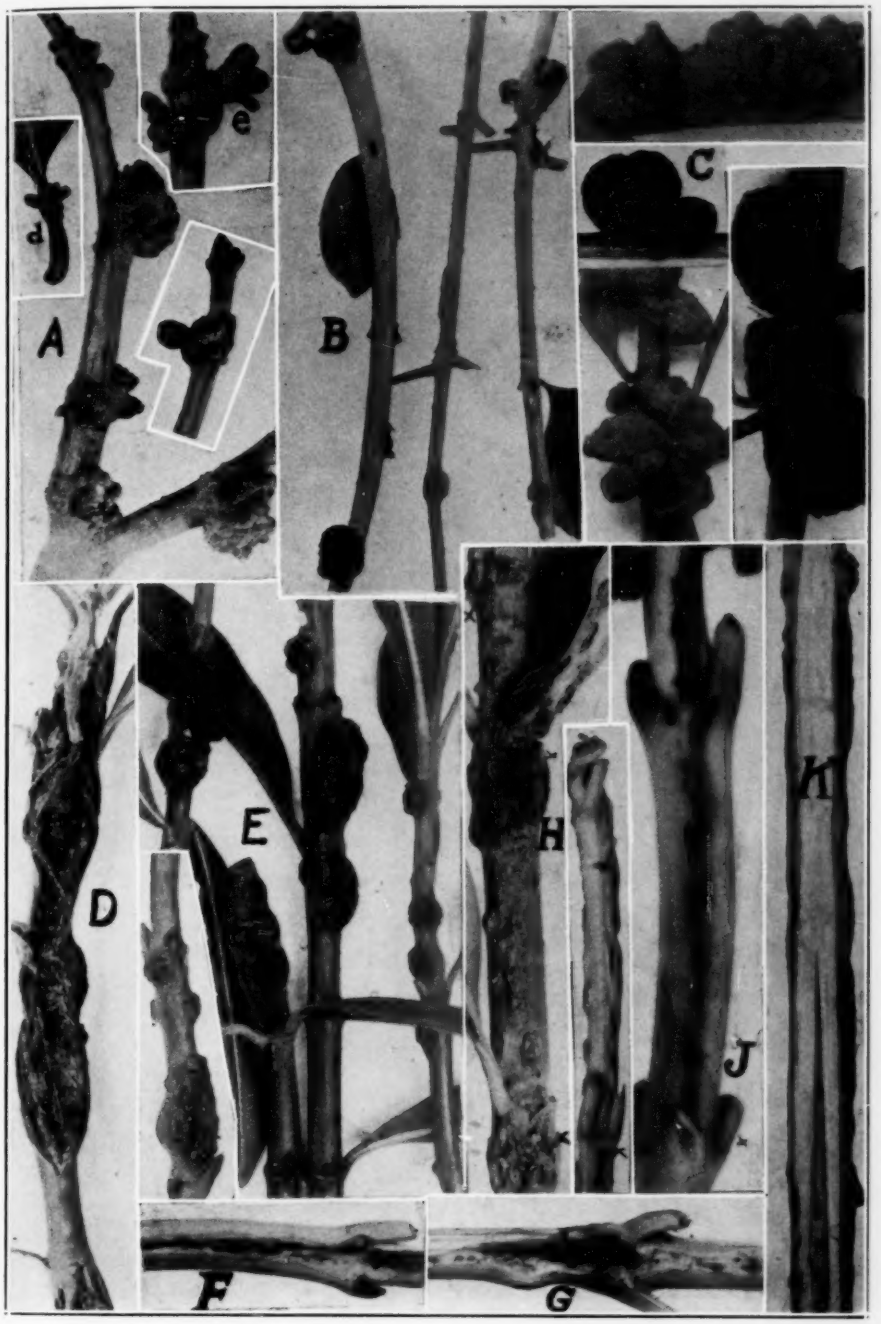
PLATE IX

Artificial inoculations and sections of diseased tissues. A: Two primary inoculations on oleander and infection channel connecting them and extending into the

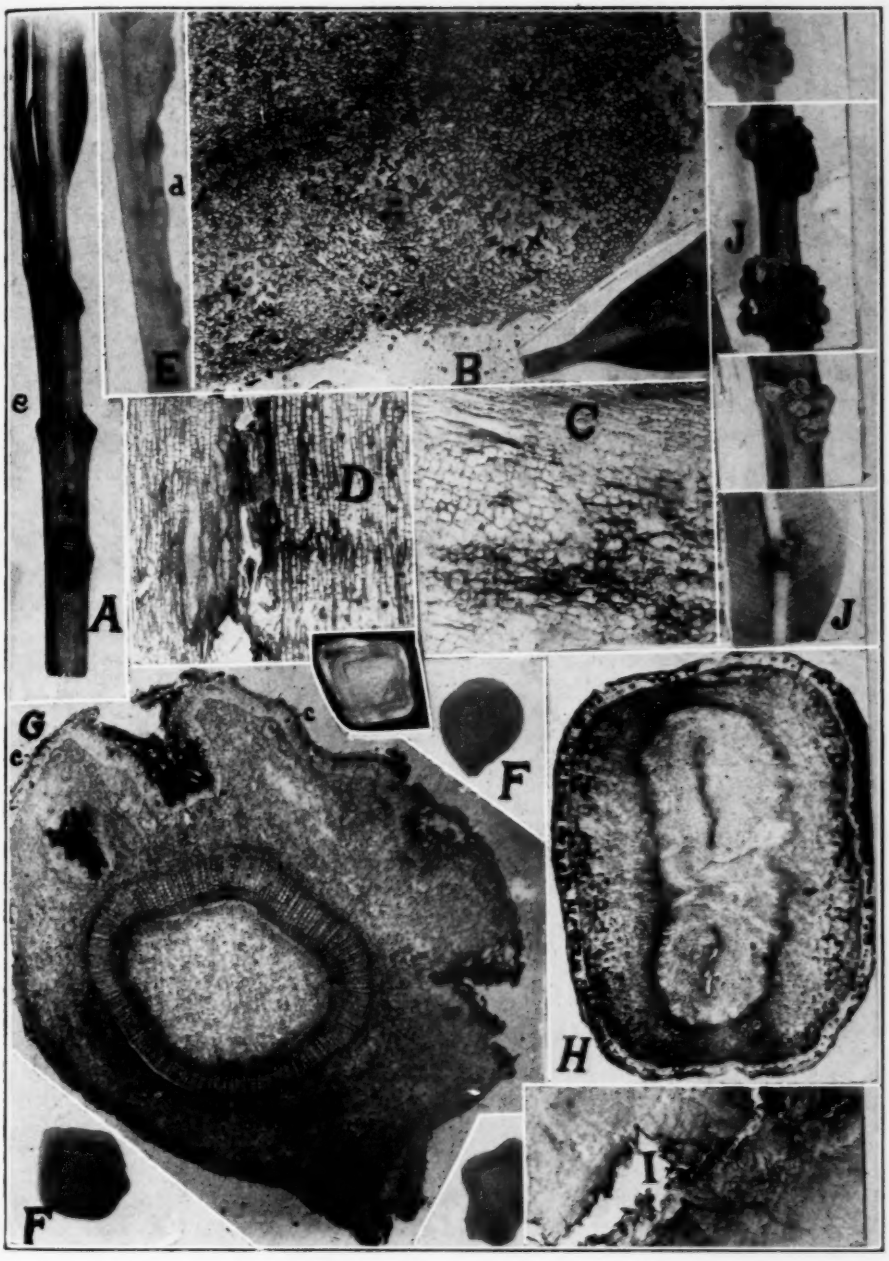
leaf petiole; photographed 35 days after inoculation. B and C: Cross and longitudinal sections of stem in A; made above the node at e; note in B the five bacterial areas at x, and the broken-down tissue in C. D: Longitudinal section through areas of bacteria in an infection channel similar to A from an inoculation made at the same time with a different culture. E: An older infection channel through which sections were made at d showing blackened areas as seen in F. G: A cross-section of F magnified to show bacterial areas in cortex. Other initial bacterial areas are shown at G, c.

H: Section of artificially produced knot in *Chionanthus* (Plate VIII, A, e). I: Section of an artificial knot of olive produced by the oleander organism. J: Infection on oleander with *Ps. tumefaciens*. Note above upper gall two small tumefactions.


A
B
A
B
C
D
E
F
G
H



UNIV. OF MICH.



UNIV. OF MICH.

DRY ROT OF GLADIOLUS CORMS

L. M. Massey

The term "dry rot" was first used by Wallace (9), who recognized four diseases of gladiolus corms, namely, hard rot, dry rot, soft rot, and scab. Although Wallace states (9, p. 69) that he was probably including two distinct diseases under the name "dry rot," his brief description of one of the two fungi, isolated from corms from Germany and New York State, leaves no doubt as to the identity of the disease with the one here described. With the termination of the study by Wallace, Fitzpatrick (3) gave some attention to gladiolus diseases and throughout his study restricted the term dry rot to the disease as it is now known. The writer (4) adopted the terminology of the above-mentioned workers, as did Drayton (2) in presenting the most extensive account of the disease thus far published.

Although mention of gladiolus diseases is to be found in several publications antedating the articles cited here, the meager descriptions of both disease and pathogene and the absence of infection experiments create doubt as to what diseases were involved. Wallace (9, p. 14) states that "the disease reported by Robinson (8, p. 139) is probably identical with that which we are calling dry rot, specimens of which were received from Germany and also from New York State. At least the presence of a fungus with Rhizoctonia-like mycelia in the tissue suggests this." Aside from this doubtful instance the dry rot disease can not be identified in literature prior to 1909.

Dry rot probably occurs generally wherever gladioli are grown. Hundreds of specimens have been received from growers in the United States and Canada and the disease has been found on corms from England, France, and Holland. Dry rot, hard rot (5), and neck rot (7) are the most common and prevalent diseases of the gladiolus, a plant which has greatly increased in popular favor and economic importance during the past two decades. The losses from these three diseases are large, especially in years of high precipitation and when there is unfavorable weather at the time of harvesting the corms.

SYMPTOMATOLOGY

All parts of the plant below ground may be infected, and a stem-rot at the surface of the soil involving the lower inch or two of the stem may

often be observed. This rotting of the stem frequently results in a premature yellowing of the tops, followed by drying and death. The plant may or may not bend at the rotted area, depending on the extent of decay, which in turn is dependent on the amount of moisture present. Usually in cases of stem-rot the corm will be found to be affected, but instances may be observed in which the corm appears quite free from lesions. It is possible that in these cases the husks or sheathing leaf bases of the parent corm are diseased. In stem-rot the outer sheathing leaf bases are characteristically attacked first. Minute, black sclerotia of the pathogene are usually present on diseased stems.

As in the case of hard rot (5, p. 157), but less frequently, yellowing of the foliage and death of the plant may result from the decay of the parent corm affected with dry rot when planted. This usually happens about midseason when temperatures are high and the soil dry. Saprophytic fungi are frequently involved in the final destruction of the old corms, although the dry rot advances more rapidly under the favorable environment of the soil and may of itself cause the death of the plants.

The dry sheathing leaf bases or "husks" of stored corms affected with dry rot may show evidences of disease by being abnormally dark in color and brittle. These darkened areas are usually irregular in outline, rarely being approximately circular as are the lesions on the corms. There is a tendency for the affected husks to split longitudinally, and pieces may be broken away easily, exposing the corm beneath (Plate X, B). The husk lesions may or may not be immediately over lesions on the corms. In most cases it is necessary to remove the husks to determine whether or not a corm is diseased.

On removing the husks it will be found that diseased corms characteristically bear many small lesions which range in size from mere dots to areas about one centimeter in diameter (Plate X). Larger individual areas are relatively uncommon, but are to be found and may involve half or more of the corm. Frequently, numerous lesions coalesce to form a large lesion, which in advanced cases may involve the entire corm and reduce it to a dry and shriveled mummy. When this condition exists it is usually still possible to trace the outlines of individual lesions. Areas more or less healthy may be left insulated in large areas of diseased tissue. Not uncommonly the lesions occur along the juncture of the husk and the corm, forming rings of diseased tissue. This would seem to indicate that the husk was first affected, the rot spreading from the husk to the corm. On the other hand, diseased corms with apparently healthy husks are commonly found.

The individual lesions are approximately circular in outline and appear first as minute, reddish brown spots, usually on the side and lower half of the corm but not infrequently on the upper half as well. The line of

demarcation between healthy and diseased tissue is rather sharp. As the lesions increase in size, the centers become sunken, the color usually deepens to black, and the margins become more definite. The sunken centers of the lesions probably result from the drying of the tissue. The margins of the lesions, especially noticeable in the larger and older diseased areas, are slightly elevated. The consistency of diseased tissue is characteristically corky.

As a rule, the lesions do not extend deeply into the corm, the range being from 1 to 5 or 6 millimeters and the average less than 3 millimeters. Usually the diseased tissue can be separated readily from the apparently healthy tissue beneath. This symptom is not restricted to the dry rot disease but is at least equally true of hard rot, Fusarium rot, and scab. Evidently, in this stage of the advancement of the disease, the corm has the advantage in being able to callous over the diseased area and, for a time at least, prevent further advance of the invading hyphae.

A more detailed study of the larger and older lesions will sometimes disclose the presence of minute black bodies buried in the diseased tissues. These are the sclerotia of the pathogene. They are sometimes present on the husks (Plate X, E) but are not found in a sufficiently high percentage of instances to be of much importance as an aid in diagnosis.

Cormels produced by diseased plants are commonly affected. At the time of harvesting the corms it is frequently possible to distinguish diseased cormels by their darker color, but this difference in color is less marked on drying, if at all evident. The lesions to be found by removing the hard outer coats are similar to those found on the corms, and mummification during storage results in a high percentage of cases.

ETIOLOGY

The dry rot of gladiolus is caused by the fungus **Sclerotium gladioli** n. sp.

The pathogene is readily obtained in culture by the usual methods of making isolations from infected tissue. It is a relatively rapid grower and will tolerate a wide range of media and cultural conditions. As stated below, growth varies somewhat with different media, but the variations are not great enough to render difficult the identification of the fungus.

Sclerotia similar to those produced on stems and husks, and sometimes within the infected tissues of the corms, are readily formed in culture in from 10 to 14 days (Plate XI). Measurements of 100 sclerotia produced on potato agar[1] in a petri dish after three weeks at about 20° C. gave a diameter

[1] In preparing the potato agar 200 grams of potato were used per litre, to which were added 20 grams of agar. For cornmeal agar, 50 grams of cornmeal were used per litre, with 2 per cent agar. Media did not contain sugar unless indicated.

range of 90–300 × 90–240 μ, with an average of 191 × 164 μ; on potato agar in a petri dish, 27 days old, at about 20° C., a range of 125–261 × 125–208 μ, with an average of 188 × 161 μ; on cornmeal agar[1] in a petri dish, 27 days old, at about 20° C., a range of 115–229 × 105–208 μ, with an average of 182 × 159 μ; and for 100 sclerotia formed on the stem of a diseased plant (natural infection) growing in the greenhouse, measurements showed a range of 94–156 × 73–135 μ, with an average of 119 × 106 μ. Drayton (2, p. 206) gives the range as 187 × 156 to 93 × 93 with an average of 158 × 118 μ, the source of the sclerotia not being given. Evidently they vary somewhat, depending on conditions under which they are formed.

The sclerotium is black. It consists of a definite cortex of thick-walled cells, from one to two cells in width, surrounding thin-walled parenchymous cells (Fig. 1, A). On crushing a sclerotium, small globules of an oily substance are exuded. Although growths are obtained from planting sclerotia on artificial media in petri dishes, there is no evidence that the sclerotia, and not attached or adhering hyphae, initiate the culture.

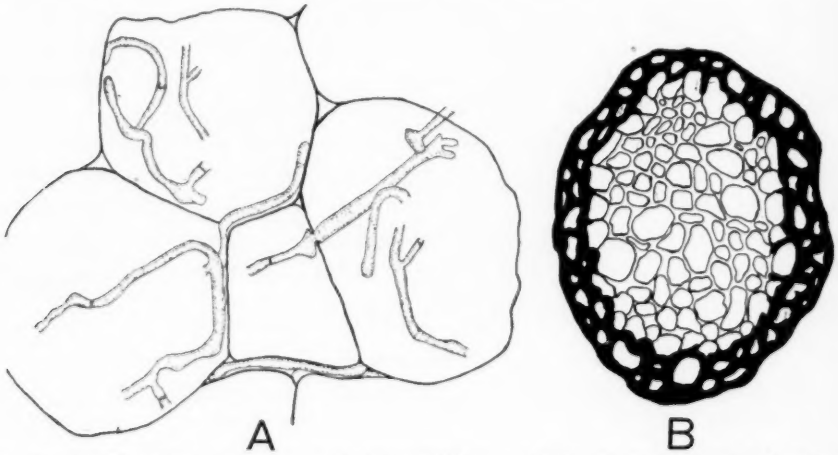


FIG. 1. A, Mycelium is inter- and intra-cellular. × 350. B. Section of sclerotium, from culture on potato agar, showing definite cortex and parenchymous medulla. × 300.

The only spores produced appear to be microconidia. These have been observed only in culture. These microconidia were first found in a culture of the fungus on a synthetic medium,[2] in test tubes, and later on potato agar, especially when adjusted to a pH of about 5 by the addition of hydrochloric acid. The cultures in which microconidia have been found

[2] Water 1000 c.c., agar 15 grams, glucose 20 grams, peptone 10 grams, dipotassium phosphate 0.25 gram, magnesium sulfate 0.25 gram, as given by Cook and Taubenhaus (1).

were from 25 to 40 days old. Small white granules, less than a millimeter in diameter (Plate XI, D), appear buried in the medium mostly at the top and back of the slant, adjacent to the wall of the tube. On examination under the microscope these granules are found to consist of numerous spores, borne on verticillately branching conidiophores (Fig. 2, A). The microconidia are approximately spherical, 1.7–3.7 μ in diameter, with an average of 2.5 μ. Attempts to germinate them have failed, and, since they have not been found under natural conditions, the role they play, if any, in disseminating the fungus and in infection is unknown.

The mycelium is septate, and both inter- and intra-cellular. On media used by the writer it is white in color, with the aerial mycelium tipped with clay[3] in older cultures.

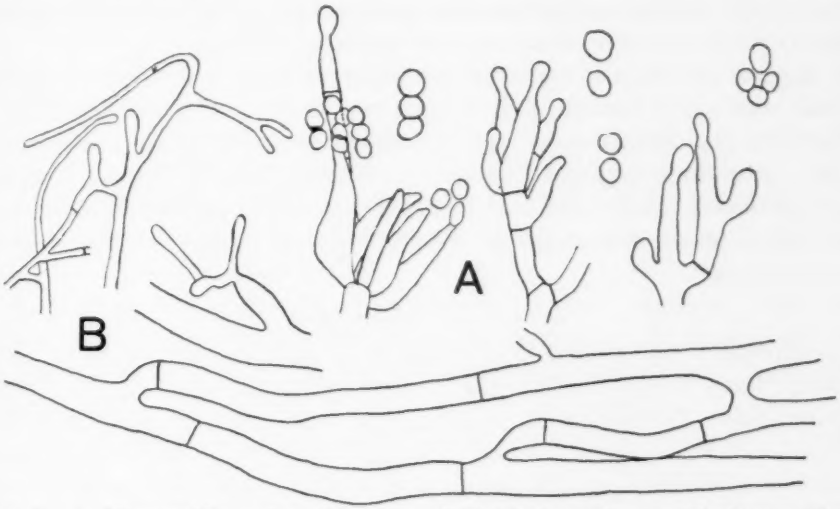


FIG. 2. A, Microconidia and portions of verticillately branching conidiophores. From a 40-day-old culture on potato agar, pH 5.04. ×1500. B, Mycelium from a 7-day-old culture on potato agar to show manner of branching. ×500.

Since no spore stage other than microconidia has been found, it seems necessary to continue the fungus in the form genus *Sclerotium* as was suggested by the writer in 1918 (4, p. 72). Because of the structure of the sclerotia the form genus *Sclerotium* is preferred to *Rhizoctonia*, even though the method of branching of the mycelium suggests to a certain extent that of the latter genus (Fig. 2, B). The species name **gladioli** is here proposed. The formation of sclerotia and microconidia, of the type figured (Fig. 2, A), strongly suggests that the fungus may be a species of *Sclerotinia*, but at-

[3] Designations of color are according to Ridgway, Robert. Color standards and color nomenclature. 44 pp., 53 col. pl. Washington, D. C. 1912.

tempts to bring about further development of the sclerotia have been unsuccessful.

PATHOGENICITY

The ability of *Sclerotium gladioli* to infect the gladiolus and produce symptoms identical with those found under natural conditions has been established through numerous experiments both in the field and under greenhouse conditions. Wallace (9, p. 72), Fitzpatrick [according to Whetzel (10)] and Drayton (2, p. 205) report successful infection experiments. Infection takes place when corms are planted in infested soil, and when the progeny of healthy corms planted in clean soil are inoculated by placing mycelium in contact with them. Sound corms placed in sterilized moist sand in moist chambers and inoculated by pressing bits of medium carrying the fungus against the uninjured surface, when held at a temperature of 25° C., develop lesions in about ten days.

A phellogen layer is formed at the juncture of the diseased and healthy tissue, similar to that formed in hard rot (5, p. 168). Diseased tissue, especially in advanced cases, can be severed from the apparently healthy tissue beneath, the rupture occurring at this phellogen layer. Sections through lesions show from one to many layers of these thin-walled cells indicating progressive stages in the advancement of the fungus into the healthy tissues.

CULTURAL CHARACTERS

Sclerotium gladioli has been grown on a large number of media, both standard and special, the latter being used largely in an attempt to induce sporulation. The fungus is tolerant of a wide range of conditions, and growth is fairly uniform. The mycelium is mostly white, at first appressed and somewhat silky, later becoming more distinctly aerial (in petri dishes) in the outer one-third or so of the growth. The surface of the medium in a standard petri dish is covered in about 7 days, with appressed mycelium in the inner two-thirds of the diameter and aerial mycelium forming a ring covering the outer one-third or so of the surface. Later the area of appressed mycelium is increased somewhat, but the ring of aerial mycelium near and on the wall of the dish persists indefinitely. With age the color of the aerial mycelium is gradually changed from white to clay.[3] Sclerotia appear in from 5 to 20 days, usually in the older parts of the culture following a darkening of the mycelium, although in some instances, *e.g.*, on cornmeal agar without sugar, they appear simultaneously throughout the culture and with no darkening of the hyphae (Plate XI, F).

The growth on oat-mush agar is more rank than on decoctions of potato and cornmeal, growth on the latter being scanty and relatively inconspicu-

ous. Sugars, 2 and 5 per cent, added to plant decoctions increase the growth rate slightly and hasten the formation of sclerotia (Plate XI, E, F). Fructose was found to be slightly better than dextrose and saccharose for sclerotial formation, and 2 per cent as effective as 5 per cent although the growth rate was somewhat increased with the larger amounts. With sugars added to decoctions of potato, cornmeal, and oats, a sclerotial crust is formed, while without sugars the sclerotia stand out as individuals. Zonation is sometimes present in cultures in petri dishes, but is not very conspicuous. Lactic acid, two drops to 15 c.c. of medium, slightly retards sclerotial formation, although the fungus is more tolerant toward a neutral or acid medium than toward one that has been made alkaline by the addition of sodium hydroxide. Saltations result frequently, especially where but a thin layer of medium in petri dishes is available to the fungus.

Cultures of *Sclerotium gladioli* have a very pronounced musty odor which may aid in the identification of the fungus. This odor is strongest in recently isolated cultures, becoming weaker as the growth ages. On several occasions recent isolations of the fungus have either failed completely or made irregular growths when cultures have been confined in closed containers of limited volume. Whether or not there is any relation between these failures and the presence of the substance responsible for the characteristic odor has not been established.

Reaction between cultures from different sources. Among other attempts to induce sporulation by *Sclerotium gladioli* two or more isolations of the fungus were grown at one time in the same petri dish. Although these attempts were failures so far as fruiting is concerned, it was found that certain isolations, when paired, resulted in the formation of a heavy dark line at the junction of the thalli, whereas other combinations did not react in this manner (Plate XI, A, B, C). It was thus possible to divide the cultures from numerous sources into groups, positive and negative in this respect, no two members of either group reacting with each other. The full significance of this characteristic, and whether or not anything other than the question of staling is involved, is not known.

Temperature relations. Cultures of *Sclerotium gladioli* were established by transferring bits of a solid medium carrying mycelium to the center of petri dishes of equal size containing equal amounts of hard potato agar (3 per cent) with 1 per cent dextrose. The medium was tested electrometrically and found to have a pH value of 7. After incubating all of the cultures for 24 hours at 20° C., they were divided into lots of four dishes each and each lot held for 10 hours at the particular temperature at which it was to be kept throughout the experiment. This permitted the fungus to establish itself in the medium in the petri dish and eliminated errors that might

arise from failure to allow sufficient time for temperature adjustments before making initial measurements. The diameters of the thalli were then recorded and the rate and amount of subsequent growth determined by measuring the increases over the original diameters. Four cultures were placed in each of a series of temperature chambers with the following temperatures: 0°, 5°, 10°, 15°, 20°, 25°, 27.5°, 30°, 32°, and 35° C. The diameters of the thalli at 72 hours were taken as a criterion for comparison, the results being represented graphically in figure 3. There is a gradual increase in rate of growth up to the optimum at 25° C., with a rapid drop beyond 27.5° C.

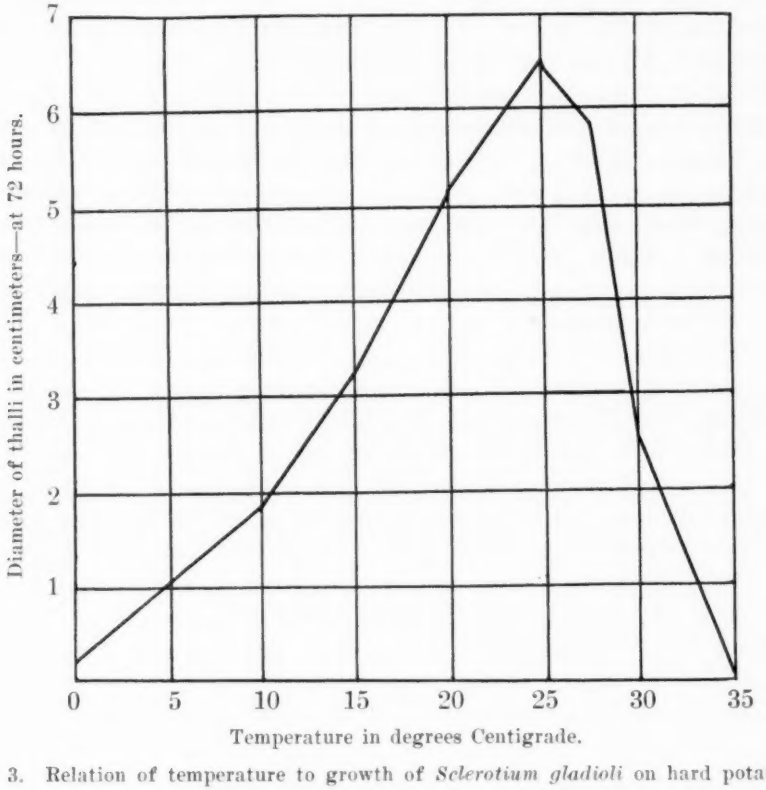


FIG. 3. Relation of temperature to growth of *Sclerotium gladioli* on hard potato agar with 1 per cent dextrose in petri dishes.

LIFE HISTORY

The dry rot fungus may be isolated from lesions on the corms at any time during winter or spring. This shows that the living organism is carried to the soil along with diseased corms at planting time. About 30 per cent of the offspring from diseased corms planted in clean soil are infected. The fungus does not grow directly from the old into the new corm. It either grows through the sheathing leaf bases or else out into the soil, from which

it attacks the newly developing corm. In connection with inoculation experiments it was observed that the fungus freely enters the soil, and experiments previously reported (5, p. 162, 180) show that the fungus will live in soil in which no gladioli have been grown for at least four years.

The fungus, then, passes the winter in diseased corms, in infested soil in the field, and doubtless also to some extent in infected stems. Just what rôle is played by the sclerotia, found especially on husks and the lower parts of the stems, is unknown. Further, since the microconidia have not been germinated, the part they play, if any, in the life history of the fungus remains unsolved. The fact that diseased corms almost always show numerous lesions suggests the possibility of infection by spores, but no evidence to pathogene, approximately 70 per cent of the progeny will escape infection. since sound corms planted in soil in which no gladioli have been grown have uniformly remained sound and produced disease-free offspring.

As previously reported (5, p. 173), hundreds of healthy corms have been housed over winter with many thousands of diseased corms without becoming infected. This indicates that *Sclerotium gladioli* is not disseminated in storage. It has been observed, however, that the percentage of infected corms is increased when considerable soil from the field is taken into storage on improperly harvested corms. The excess soil holds moisture, delaying drying of the husks, and under such conditions the fungous mycelium may actually grow from corm to corm. Lesions increase in size during storage especially where the temperature and humidity are high.

CONTROL

In connection with an investigation of the hard rot disease the writer included corms affected with dry rot in the several experiments on control measures (5, p. 172–180). The treatments of diseased corms with (*1*) formalin at the rate of 1 pint of commercial formalin to 15 gallons of water for 18 hours, (*2*) corrosive sublimate, 1–1000 solution, for 18 hours, or (*3*) chemicals, including sulfur, air-slaked lime, acid phosphate, and soot, in which the corms were rolled and with which they were covered after placing in the row and before covering with soil, were found to be of no value. These treatments were made in the spring before planting. Similar tests were made in the autumn immediately after digging, at which time the lesions are materially smaller than in the spring. Failure again resulted. Recently dug corms have also been treated with formaldehyde gas (5, p. 177), with water at 50° C. for one-half hour, and with dry heat at 50° C. for one and one-half hours. These treatments were unsuccessful in reducing the percentage of diseased corms, and the dry-rot fungus was isolated from numerous lesions, showing it to be viable still. Drayton (2, p. 208) reports

failure in the use of formalin, mercuric chloride, Uspulun, and Bayer compound, both for the treatment of diseased corms and as a drench for soil in which diseased plants had grown. No satisfactory material for the disinfection of soil has been found.

When corms affected with dry rot are planted in soil free from the pathogene, approximately 70 per cent of the progeny will escape infection. It is evident, then, that soil infestation is one of the most important factors to be considered in connection with control measures.

In an article on Fusarium rot of gladiolus corms (6) the writer has proposed general recommendations for control, involving sorting, crop rotation, proper harvesting and storage, which are applicable to the several corm rots, including dry rot.

SUMMARY

Dry rot is an important disease of gladiolus corms in the United States and Canada, and is known to occur in England, France, and Holland. A description of the disease is given. Corms and cormels become infected in the field, and the rot advances in storage. Plants in the field may die during the season either from a stem rot or the decay of the corms. Soil becomes infested from planting diseased corms, and the pathogene will survive in the soil for at least five years.

The pathogene causing dry rot is placed in the genus *Sclerotium* because of the structure of the sclerotium and the absence of spores other than microconidia. A new species, *gladioli*, is made. The morphological and cultural characters of the fungus are recorded.

On hard potato agar with 1 per cent dextrose, the fungus grows over a range of from about 0° to 35° C. Optimum growth takes place at about 25° C.

Infection was readily obtained on uninjured corms in the laboratory and on those growing in the greenhouse and experimental gardens.

No satisfactory treatment of diseased corms to disinfect them has been found. Crop rotation, avoiding the use of the same soil oftener than once in four or five years, is believed to be of major importance in holding dry rot in check. This practice should be combined with sorting to eliminate diseased corms, together with the proper handling of corms at harvest and during storage.

CORNELL UNIVERSITY,
ITHACA, N. Y.

LITERATURE CITED

1. COOK, M. T. and J. J. TAUBENHAUS. The relation of parasitic fungi to the contents of the cells of the host plants. Dela. Agr. Exp. Sta. Bul. 91. 1911.

2. DRAYTON, F. L. The dry rot disease of gladioli. Scientific Agriculture **6**: 199–209. 1926.
3. FITZPATRICK, H. M. Gladioli bulb rots. The Florists' Exchange **32**: 455–456. 1911.
4. MASSEY, L. M. Dry rot of gladiolus (Abst.). Phytopath. **8**: 71–72. 1918.
5. ————————. The hard rot disease of gladiolus. N. Y. (Cornell) Agr. Exp. Sta. Bul. 380. 1916.
6. ————————. Fusarium rot of gladiolus corms. Phytopath. **16**: 509–523. 1926.
7. MCCULLOCH, LUCIA. A leaf and corm disease of gladioli, caused by *Bacterium marginatum*. Jour. Agr. Res. **29**: 159–177. 1924.
8. ROBINSON, W. The English Flower Garden. CXXIV + 332 pp. John Murray, London. 1883.
9. WALLACE, ERRETT. Some bulb rots of gladioli. Unpublished thesis, Cornell Univer. 1909.
10. WHETZEL, H. H. Gladiolus disease investigations. Florists' Exchange **32**: 1035. 1911.

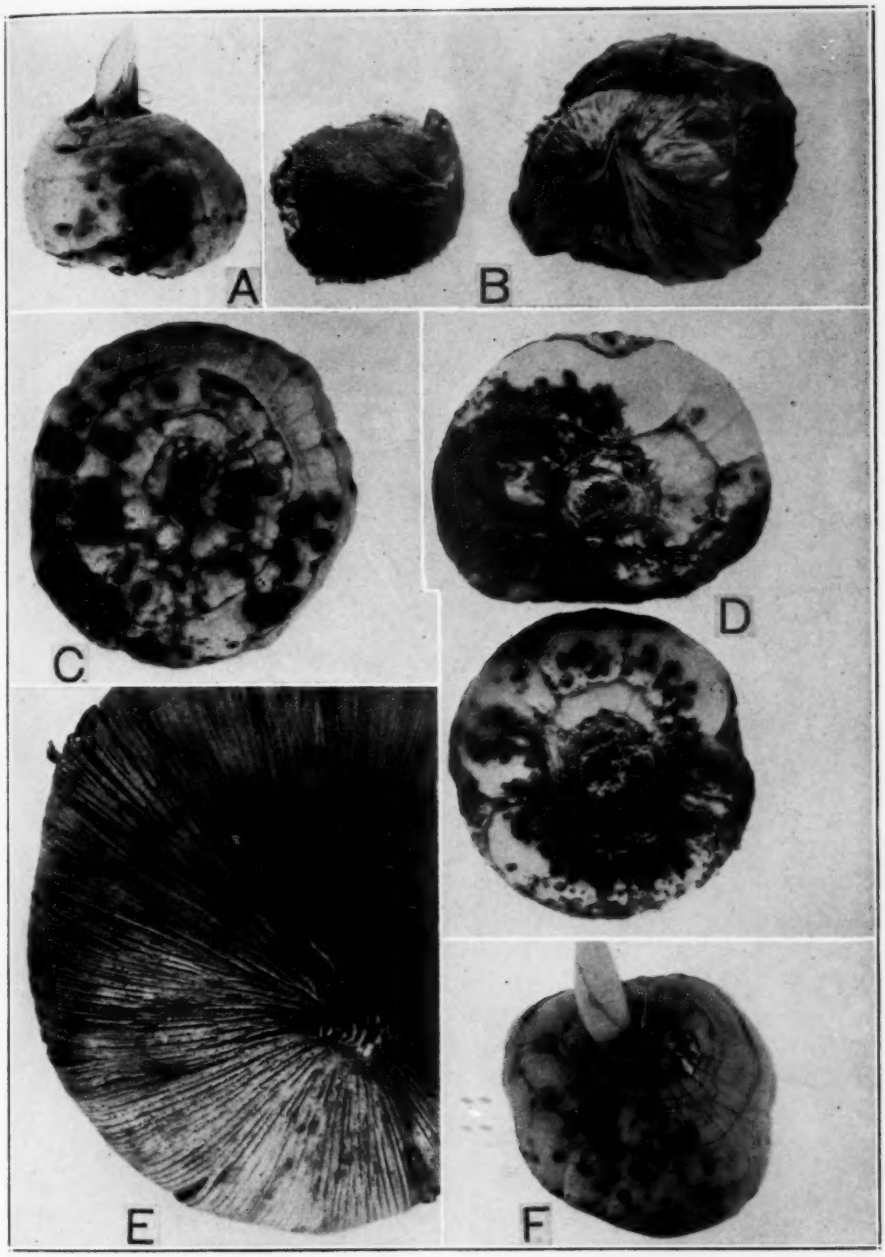
EXPLANATION OF PLATES

PLATE X

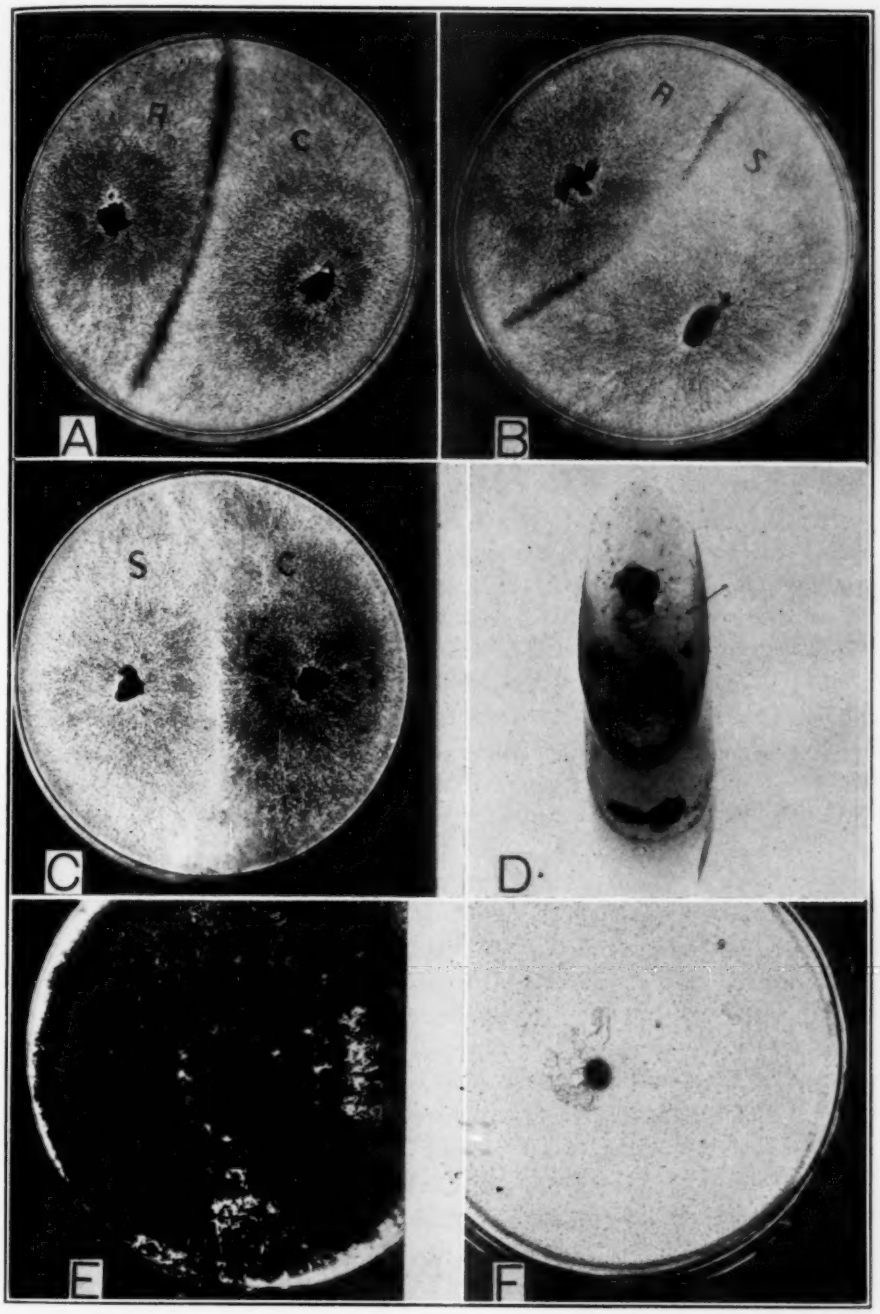
Corms affected with *Sclerotium gladioli*. Corms at lower left and upper right show sclerotia on husks. A, B, C, D, and F, approximately natural size; E, ×2.

PLATE XI

Cultures of *Sclerotium gladioli*. A, B and C show results of growing paired isolations from different sources on potato agar in petri dishes. Note reactions, indicated by dark-colored junctures, between A and C, and A and S. Photographed after 10 days. D, Microconidia produced on potato agar, after 40 days. Aggregations of spores and conidiophores appear as small, globose, granules, white by reflected light, buried in the medium, as indicated by arrow. E, F, cornmeal agar with and without 2 per cent dextrose, respectively, after 40 days. Note effect on formation of sclerotia.



UNIV. OF MICH.



UNIV OF MICH.

"BLACK TIP," A FINGER-TIP DISEASE OF THE CHINESE BANANA IN BERMUDA

LAWRENCE OGILVIE

INTRODUCTION

The "Chinese," "Canary," "dwarf," or "Cavendish" banana (*Musa cavendishii* Lamb), introduced into Bermuda probably about 1850, has furnished an important article of food since that time. It is grown, however, only for local consumption since the cost of production is excessive compared with similar costs in other banana-producing countries. At the present time there are about 50 acres of the more sheltered land in the valleys under cultivation in bananas, which produce some 35,000 bunches a year.

In the Annual Report of the Board and Department of Agriculture, Bermuda, for 1924 (7), reference is made to a somewhat serious disease, commonly known as "black tip," which was first brought to the notice of the writer in August of that year. There is evidence that the disease had been present in the Islands for a considerable number of years before that date but up to then had occasioned little loss.

Under Bermuda conditions the flowers of the female fingers of the Chinese banana either fall off, a break occurring at the base of the perianth segments and leaving a scar which rapidly dries up, or wither and remain attached to the fruits. The bunches are gathered when they are still green but when the fingers have become well filled.

SYMPTOMS

Under certain conditions a black discoloration becomes apparent just below the flower and progresses down the fruit. In about three weeks' time it may progress some two inches down from the tip. The diseased area is frequently irregular in outline, often affecting one side of the fruit more than another, and is bordered by a narrow grey or light yellow margin. An ill-defined zonation is frequently present. (Fig. 1.)

ASSOCIATED ORGANISMS

Investigations carried out in 1924 and at subsequent intervals when time was available show that a large number of organisms are commonly present on the withering flowers. These include species of *Fusarium* and *Cephalosporium*, *Aspergillus luchuensis*, Inui (identified by Dr. Charles

Thom), *Gloeosporium musarum* Cooke and Massee, species of *Macrosporium* and *Alternaria*, and bacteria. The whole flower is frequently covered by the frass and silky webs of a Tineid larva. Predatory mites of the genus *Hypoaspis* (*Gamasidae*) are commonly found on the withering flowers and

FIG. 1. A bunch of the Chinese banana severely damaged by the "black tip" disease. The fruits were still green when the photograph was taken.

fruit tips. Other organisms which growers supposed might be implicated in the diseased condition are snails, chiefly *Polygyra appressa* Sag., very commonly found on the tips of the fruits, especially after rain, and the aphis *Pentalonia nigrinervosa* Coq., which clusters on the fruit-tips and is attended by ants. The exudation of latex, if the break occurs when the flower is still unwithered, also attracts large numbers of Drosophilid flies, chiefly *Drosophila ampelophaga* Loew. (See 8.)

It was soon evident, however, that none of the organisms other than the fungi were invariably present, except the mites, and these were found to be present on healthy and diseased fruits. With regard to the fungi and bacteria, inoculations of all those isolated in 1924 and again in 1925 produced no marked effect on green banana fruits.

A further outbreak of the disease in the summer of 1927 again drew the writer's attention to it; and further investigations showed that at a relatively late stage, when the discoloration had progressed about half-an-inch down the fruit, pale brown mycelial wefts were present on the surface of the fruit, extending to within about 3 mm. of the margin of the lesion, though at advanced stages they tended to be obscured by thick growths of *Cephalosporium*, *Fusarium*, and other fungi. Little difficulty was found in isolating this fungus, which proved to be identical with *Cercospora musarum* Ashby, first described by S. F. Ashby from Jamaica (3), where it was found to be associated with a "blackspot disease" of the leaves of the Gros Michel banana.

FRUIT INOCULATIONS

A large number of constantly successful inoculations with this fungus were carried out during August, 1927, both on detached fruits and on bunches in the field. Mycelium from pure cultures grown on 1 per cent dextrose potato agar produced a rapid discoloration when placed in small incisions in the skin of green bananas, the surfaces of which had been previously sterilized. Check inoculations remained normal. In less than two days after inoculation a black discoloration extended outwards from the point of inoculation, and in about three weeks a lesion some two inches in length was produced (Fig. 2). The successful results of these inoculations were referred to in a short note published in the Bermuda Agricultural Bulletin (6). The fungus has been reisolated constantly from these lesions. None of the inoculations with the other fungi was successful in producing the characteristic discoloration, although an *Alternaria* produced a slight surface rot.

SECONDARY ORGANISMS.

The whole fruit is seldom affected. The ripening of the fruit appears to put a stop to the progress of the lesion. At this stage, however, the skin

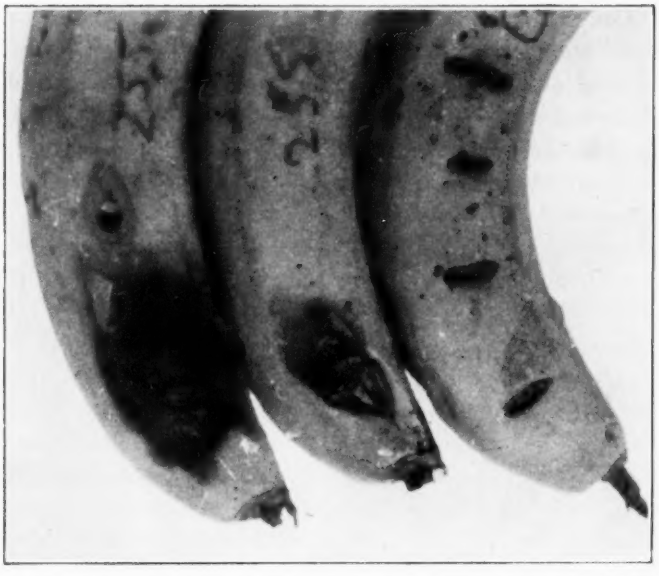


FIG. 2. Green fruits inoculated with *Cercospora musarum* Ashby. Cheek incisions at the left. Photograph taken a fortnight after inoculation.

of the affected area tends to split, and numerous insects are thereby attracted. The larvae and adults of Carpophilus beetles (*Carpophilus dimidiatus* Fabr., etc.), of *Araecerus fasciculatus* de Geer, and of Drosophilid flies are especially frequent. *Chaetopsis aenea* Wd. (Ortalidae) has been bred out from such fruits. These and other insects, and also snails and rats, soon render the affected bunches quite unsalable.

THE FUNGUS

The fungus in question has been well described by Ashby in his account already referred to (3) and quoted by Nowell in his book on "Diseases of Crop Plants in the Lesser Antilles" (5). Nowell is at present carrying out a further investigation of the fungus with a view to determining its systematic relationships. It grows well on 1 per cent dextrose potato agar, producing a somewhat flocculent white mycelium which becomes dark olive green with age. In plate cultures the aerial mycelium produced from the centers of the colonies is a characteristic feature. Recently isolated cultures sporulate abundantly at summer temperatures (about 75° F.).

Microscopic examination of the affected areas of the fruit shows that the fungus is confined mainly to the green skin, where it brings about necrosis of the cells. Its hyaline to fuscous intercellular mycelium some 4 μ in

diameter may be readily seen in sections. The spores of the fungus are produced in abundance on the surface of the lesions during warm weather.

SYMPTOMS ON THE LEAVES

The fungus *Cercospora musarum* is associated with two distinct types of lesions on the leaves of the Chinese banana:

1. Minute round black spots scattered over the surface of the leaves and especially in the neighborhood of the midrib. These are probably identical with those referred to by Ashby as "pin-point round black spots on the main nerves of the leaf blade." The fungus has been isolated constantly from such spots, and conidia of the fungus are produced in abundance on the surface of such areas if pieces of the leaf are kept in a moist chamber.

FIG. 3. A typical leaf-lesion caused by *Cercospora musarum* Ashby.

2. Large brown lenticular lesions surrounded by a bright yellow margin (Fig. 3). Such areas usually commence at some point of injury, very frequently at the edge of a leaf blade or where it has split transversely, or from the mottled areas associated with a *Phyllosticta* (probably *Phyllosticta musae-sapientii* Frag. et Cif.). The fungus has been isolated constantly from the edges of such lesions. A surface mycelium identical with that found on the fruits is frequently found on such areas, as is also the characteristic zonation associated with the tiprot of the fruits. If pieces from such areas are kept in a damp chamber, an abundant crop of conidia may

be secured. It should be mentioned, however, that, during the winter months in Bermuda and under other conditions unfavorable to the growth of the plant, dead areas appear on the leaves which can be distinguished with difficulty from those caused by the *Cercospora*.

In his report of the "blackspot disease" in Jamaica, Ashby (3) described symptoms identical with the foregoing. He also described "round jet black somewhat convex spots with a central nipple" on fruits. These were found by him only on trees that had the black leaf spot. No such fruit spot occurs in Bermuda.

LEAF INOCULATIONS

Healthy leaves were inoculated with the fungus by cutting small nicks out of the leaf-margin and placing mycelium therein. One month from the time of inoculation (Sept. 7) distinct evidences of infection were observable on thirty such inoculated areas, while seven check wounds remained normal. Infection is first observable as black or brown streaks progressing inwards from the incised areas. Drying out of the infected areas soon takes place, the edges of the lesions being bordered by a bright yellow margin. The fungus was reisolated from the lesions without difficulty.

TEMPERATURE RELATIONSHIPS

The disease is most noticeable on both fruits and leaves during the summer months in Bermuda, late June, July, August, and September, when the mean maximum air temperature is above 75° F. By the beginning of October there is comparatively little discoloration of the tips of the very young fruits. The sales of the grower are most reduced in October and November when the greatest number of affected fruits ripen.

CONTROL MEASURES

In the light of the information obtained the following control measures have been recommended and have been followed with some success:

1. Fresh plantings should be spaced at least 8 feet by 10 feet apart in well-drained situations. As a general rule only two suckers should be left as "followers." It has been found that the disease causes most loss in old, closely planted and badly drained plantations.

2. No diseased fruits should be left lying about the fields.

3. As far as possible dead sheaths and leaves should be removed from the proximity of the bunches.

4. The large diseased spots on the leaves could be cut out with the aid of a large pruning shears, sickle, or similar tool.

Little or no control has been secured by spraying the young fruits with Bordeaux mixture to which a "sticker" was added.

SIMILAR DISEASES ELSEWHERE

A disease very similar to the black tip disease of Bermuda has been described from various localities. A *Stachylidium* is known to be associated with a tip-rot in the Canary Islands and the Gold Coast, but no successful inoculations have apparently been recorded. In the Canaries the female flowers are cut off the tips of the immature fruits since, "as they do not fall naturally, they would present an untidy appearance if left to wither attached to the fruit and would, moreover, form a harbour for insects" (1).

Mr. C. G. Hansford, formerly microbiologist in Jamaica, informs me that a tip-rot of the Chinese banana is quite common there, but has never been examined in detail as the variety is not of economic importance in that island. The occurrence of this disease in Jamaica is also referred to in an article entitled "The Banana and its Cultivation, with Special Reference to the British Empire" (2). It is said to be due to "a fungus which attacks the remains of the flower at the tip of the fruit and causes it to rot." Dr. John R. Johnston of the United Fruit Company mentions in a letter that he has noticed a similar trouble in Colombia.

Dastur (4) records the occurrence of a tip-rot of plantain in India associated with *Gloeosporium musarum*. He writes, "When the young flowers are infected, the infection is generally found to begin from the distal end, possibly arising through the flowers. The infected finger begins to turn black and shrivel from the distal end; as the infection progresses the whole finger turns black, shrivels and becomes covered with the pink spore-beds of the fungus. The attack rapidly spreads and involves the whole bunch." It was found impossible to produce lesions on green fruits of the Chinese banana by inoculations with pure cultures of *Gloeosporium musarum* under Bermuda conditions although the fungus is a common one on over-ripe fruits, thus confirming the work of R. A. Toro on this fungus (9).

It is probable that other fungi may be found to cause similar tip-rots elsewhere, but the widespread occurrence of *Cercospora musarum* on the leaves would lead us to suspect that the fruit rot would be found on the Chinese banana in localities where the fungus is present.

SUMMARY

1. A finger-tip disease of the Chinese banana (*Musa cavendishii* Lamb). commonly known as "black tip," is described from Bermuda. It is most prevalent during the summer months.

2. It has been found to be due to the fungus *Cercospora musarum* Ashby, which also causes characteristic leaf-spots.

PLANT PATHOLOGICAL LABORATORY,
DEPARTMENT OF AGRICULTURE, BERMUDA.

LITERATURE CITED

1. (ANONYMOUS). Banana cultivation in the Canary Islands. Bul. Imp. Inst. 23[3]: 168–174. 1925.
2. ————. The banana and its cultivation, with special reference to the British Empire. Bul. Imp. Inst. 22[2]: 303–333. 1924.
3. ASHBY, S. F. Banana diseases in Jamaica. Bul. Dept. Agr. Jamaica. New Series. 2[6]: 95–128. 1913.
4. DASTUR, J. F. Spraying for ripe-rot of plantain fruit. Agr. Jour. India 2: 142–148. 1916.
5. NOWELL, W. Diseases of crop plants in the Lesser Antilles. 383 pp. London. 1923.
6. OGILVIE, L. The "black tip" disease of bananas. A preliminary note. Bermuda Dept. Agr. Bul. 6[9]: 4–5. 1927.
7. ————. Report of the plant pathologist for the year 1924. Bermuda Rept. Bd. and Dept. Agr. **1924**: 32–43. 1925.
8. ————. Report of the plant pathologist for the year 1925. Bermuda Rept. Dept. Agr. **1925**: 36–63. 1926.
9. TORO, R. A. Studies on banana anthracnose. Jour. Dept. Agr. Porto Rico. 6[1]: 3–21. 1922.

HISTOLOGY OF THE LESIONS PRODUCED BY SPHACELOMA FAWCETTII JENKINS ON LEAVES OF CITRUS[1]

H. S. Cunningham

During the course of certain histologic investigations on diseased leaves, the attention of the writer was called to the condition existing in the leaves of *Citrus* affected with scab.

The lesions upon the leaves appear as hyperplastic areas somewhat lighter in color than that of the normal leaf, and have a corky appearance. The individual lesions are comparatively small but frequently coalesce to form a large corky patch. Where the lesions are numerous there may be considerable malformation of the affected leaf. The lesions are more commonly found on the under surface of the leaf although they do occur upon the upper surface as well.

The investigations reported in this paper were undertaken for the purpose of determining the histologic changes which take place in the infected leaf and which result in these characteristic warty excrescences. These studies include diseased leaves of three species of *Citrus*: rough lemon (*Citrus limonia* Osbeck), sour orange (*Citrus aurantium* L.), and Rangphur lime (*Citrus aurantifolia* Swingle). For purposes of comparison, wounded leaves of rough lemon were also studied.

Mature leaves bearing lesions were obtained through the courtesy of Dr. W. W. Yothers, of the Experimental Station at Orlando, Florida. This material was in excellent condition when received. Through the kindness of Dr. Anna E. Jenkins plants of rough lemon growing in the greenhouse at Cornell University were made available for wounding. The wounds were made on mature leaves by means of a common leather punch giving a circular hole two millimeters in diameter. In this way a clean cut wound was produced, care being taken to avoid cutting into or near the larger veins. The wounded leaves were removed from the tree after a period of eighteen days.

At the time of collection, or as soon thereafter as possible, small segments were cut from the leaves with a sharp razor. These segments included scab lesions or wounded tissue, as the case might be, together with

[1] This paper presents a part of the author's doctorate investigations on the pathological histology of leaf lesions. Grateful acknowledgement is made of the suggestions and criticisms of Professors H. H. Whetzel and A. J. Eames under whose direction the work has been done.

a portion of the healthy leaf on either side. Immediately after the segments were removed from the leaf they were placed in small shell vials containing medium chromo-acetic fixing solution (1). No vacuum pump was used to remove the air during the fixing process but the segments were kept submerged by placing a small wad of cheese cloth in the vial, thus forcing the segments below the surface of the liquid. Fixation was allowed to continue for thirty-six hours after which the material was thoroughly washed in running water and embedded in paraffin in the usual manner.

The studies were made from serial sections cut nine microns in thickness. Durand's method (2) was used in staining mycelium; crystal violet (6) for general histologic studies; and Sudan III as a test for suberin and cutin.

As the histologic changes are very similar in the diseased leaves of the three suscepts studied, it appears sufficient to present in detail the condition found in leaves of rough lemon.

REVIEW OF LITERATURE

A search of the literature revealed no satisfactory account of the histologic changes in the leaf tissue which result from attacks of the scab fungus. Numerous writers make reference to the warty appearance of the lesion and the malformation of the leaf in severe cases of infection.

Swingle and Webber (8, p. 22) state that the leaf is often considerably thickened where the wart is situated and that, as the leaves persist for at least one year, cork formation proceeds farther than in the case of the fruit. Hume (5, p. 155) says "There is often a well marked conical depression on the opposite side of the leaf corresponding to the elevation on which the excrescence is situated." Fawcett (3, p. 46) mentions the warty appearance of the scab. Grossenbacher (4, p. 134) says "When the embryonic leaves of sour-orange or grape-fruit trees begin expanding the oil-glands often protrude above the outer surface of the emerging leaves. In some cases a few of these protruding oil-glands may break open, thus giving rise to crater-like conical elevations, the upper margins of which grow more or less and tend to close the pit. In cases in which the base of such a papillum grows very strongly the former oil-gland becomes elevated on a conical growth of superficial tissues and its tip becomes covered by flaky epidermal fragments arising in what was formerly the crater-like depression of the broken oil-gland." Stevens (7, p. 84) found that as the spots enlarged they became depressed on one side and raised on the other. Winston (9, p. 12) says "Distinct hyperplasia is often in evidence beneath the area attacked by the fungus, which fact probably accounts for the plainly evident excrescence associated with the scab lesions. Specialized host tissue

can frequently be found separating invaded from uninvaded parts." This condition he found to occur in older leaves but was not observed in leaves incompletely expanded.

THE HEALTHY LEAF

In order that the reader may better understand the histologic changes which take place in the region occupied by the lesion the normal anatomy of the leaf will be presented rather fully.

In normal structure the upper epidermal cells are very regular in size and shape. They are only slightly longer than broad and are more or less rectangular in outline. The outer wall is very thick with a heavy cuticle while the radial and inner walls are also somewhat thickened. In the majority of these cells the protoplasm forms a thin peripheral layer but in others the contents are dense and granular.

The palisade parenchyma occupies approximately one half of the total thickness of the leaf, the number of cell layers varying from two to three. The cells in the two upper layers are closely packed and are considerably longer than broad while the cells of the lower layer, where such a layer exists, are shorter and broader. The cell walls are thin and the chloroplasts, while not numerous, are rather large. At intervals in the upper layer of cells and just beneath the epidermis, small cavities occur. These cavities occupy the space of two or three palisade cells and are evidently lysigenous in character.

The spongy parenchyma is comparatively open in structure and is made up of thin walled cells which are more or less oval in shape and fairly regular in outline. Each of the cells contains a large central vacuole with a layer of cytoplasm next the wall in which the chloroplasts are embedded. The cells which lie nearest the palisade layer are much larger than those lying near the lower epidermis.

The lower epidermis is similar in structure to the upper with the exception that the cells are not so thick and that some of them are more elongated. Their outer and radial walls are somewhat thickened and a relatively thin cuticle is present.

Throughout the leaf large oil-cavities are numerous. These may lie wholly within the palisade layer, wholly within the spongy parenchyma, or may occupy a portion of both of these tissues.

In the region of the larger veins only the outer layer of palisade cells is evident and these are very much shortened. The bundle proper, together with the supporting cells of thick walled parenchyma and fiber cells make up the remaining thickness of the leaf at this point.

THE DISEASED LEAF

A cross section of an infected leaf made through one of the lesions on the under surface shows a marked change from the normal at this point. This portion of the leaf is much thickened, bulging out on the lower surface with a corresponding depression on the upper surface. There has been a marked increase in the number of cells and also a marked change in the character of the cells of the tissues involved (Fig. 1).

The upper epidermal cells are apparently unchanged except that anticlinal division has taken place. The walls of the palisade cells are thickened and the chloroplasts are either reduced in number and size or have disappeared entirely. Anticlinal division has occurred and in occasional cells periclinal division as well.

The most noticeable change has taken place in the spongy parenchyma. Infection has resulted in a large increase in the number of cells as well as an increase in their size. This increase in number of cells appears to be due to division in several planes and as a result the leaf is very much thickened at this point and intercellular spaces are almost entirely wanting. In addition to the increase in number and size of cells the walls have been very greatly thickened. This thickening is entirely cellulose in nature without any indication of lignification. Many of the larger cells have been further divided by cross walls which are somewhat thinner than the main wall but of the same cellulose nature. These thick walled cells are living and each contains a peripheral layer of cytoplasm in which the nucleus is plainly visible. Early in the development of the lesion a phellogen is formed in the spongy parenchyma and as a result of the activity of this phellogen a definite phellem is laid down, thus completely isolating the portion invaded by the pathogene. The cells lying without this phellem are non-living and have suberized walls. The hyphae of the causal organism are abundant in the outer portion of this mass of suberized cells but no trace of them could be detected in the tissues lying within the phellem.

The histologic changes which take place when infection occurs on the upper surface of the leaf do not differ essentially from those already described. In such cases the phellogen is also formed in the spongy parenchyma and extends upward on either side to the upper epidermis. The phellogen thus lines a cup-like cavity filled with a mass of dead cells intermingled with hyphae. There is distinct bulging of the leaf on the under surface due to the hyperplastic condition in the spongy parenchyma.

In certain cases the phellem may extend entirely through the hyperplastic portion, thus forming a layer from the upper to the lower surface and extending as a continuous band about the edge of the lesion. Such a condition would account for the shot-hole effect sometimes accompanying this disease.

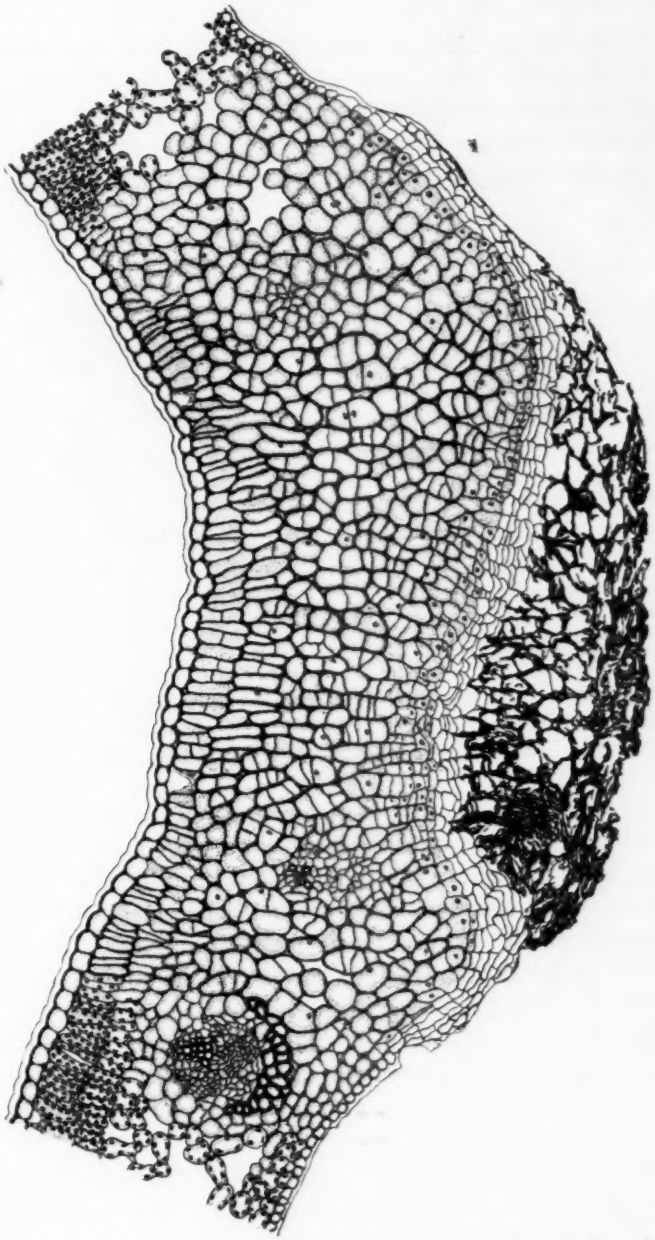


FIG. 1. A cross section through the scab lesion on a leaf of *Citrus limonia* showing the histologic changes induced by the pathogene. ×170.

THE WOUNDED LEAF

The reaction of the leaf to artificial wounding is very different from that which ordinarily takes place when infection by the scab pathogene occurs. Hyperplasia occurs, but of a very different type. A cicatrice, or band of modified cells, is formed about the wound and occupies the full depth of the leaf from one epidermis to the other (Fig. 2). This cicatrice

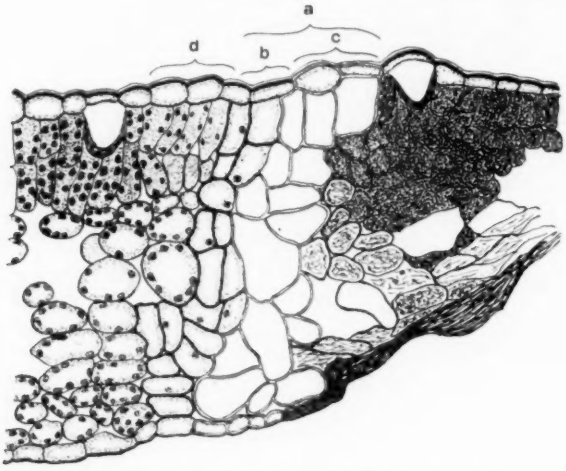


FIG. 2. Cross section through a leaf of *Citrus limonia* showing the wound periderm formed as a result of artificial wounding. a, wound periderm layer consisting of (b) phellogen and (c) phellem layers; d, modified mesophyll cells. ×300.

is formed at some little distance from the edge of the wound. The intervening cells are dead and contain a dense granular substance which resembles tannin. All of these cells, particularly those of the spongy parenchyma, are more or less collapsed, a condition which is more complete towards the edge of the wound than nearer the cicatrice.

The cicatrice may be divided into two parts: the outer part (Fig. 2, a) lying nearest the wound and consisting of a typical wound periderm with its phellogen (Fig. 2, b) and phellem (Fig. 2, c) layers; and the inner part (Fig. 2, d) lying between the wound periderm and normal tissue and in which the cells, although modified, do not show clear evidence of having originated from the phellogen.

The wound periderm consists of a layer of phellem made up of a number of large cells devoid of all contents and with heavily suberized walls. The walls of the epidermal cells adjacent to this layer are also suberized. The remainder of the wound periderm is composed of somewhat irregular shaped, thick-walled, living cells. This layer of thick-walled cells may

vary from one to several cells in width but is usually widest in the region of the spongy parenchyma. No chloroplasts are present in this layer.

In the inner layer of the cicatrice the cells vary in size, are thin-walled, and the number of chloroplasts is much reduced.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, N. Y.

LITERATURE CITED

1. CHAMBERLAIN, C. J. Methods in plant histology. 314 pp. Univ. Chicago Press. 1915.
2. DURAND, E. J. The differential staining of intercellular mycelium. Phytopath. **1**: 129–130. 1911.
3. FAWCETT, H. S. Citrus scab. Fla. Agr. Exp. Sta. Rept. **1906–07**: 45–46. 1907.
4. GROSSENBACHER, J. G. Sour scab of Citrus in Florida, and its prevention. Phytopath. **6**: 127–142. 1916.
5. HUME, H. H. Some Citrus troubles. Fla. Agr. Exp. Sta. Bul. 53: 146–172. 1900.
6. JACKSON, GEMMA. Crystal violet and erythrosin in plant anatomy. Stain Technology **1**: 33–34. 1926.
7. STEVENS, H. E. Florida Citrus diseases. Fla. Agr. Exp. Sta. Bul. 150: 15–110. 1918.
8. SWINGLE, W. T., and J. WEBBER. The principal diseases of Citrus fruits in Florida. U. S. Dept. Agr., Div. Veg. Path. Bul. 8. 1896.
9. WINSTON, J. R. Citrus scab: its cause and control. U. S. Dept. Agr. Bul. 1118. 1923.

ROOT AND CROWN INJURY TO APPLE TREES

H. E. Thomas

In an earlier paper (4) on root and crown injury of apple trees it was concluded that: (*1*) the killing of roots and crowns of apple trees in New York State is initiated in most cases by low temperatures; (*2*) certain fungi such as *Hypholoma sublateritium* Fries may aggravate the injury; (*3*) standard orchard practices and approach grafting of injured trees aid in preventing injury and in restoring trees already injured. Some additional observations and results of experiments are presented here.

RELATION OF VARIETIES OF APPLES TO INJURY

Since all varieties of apples are presumably grown on the same heterogeneous sort of stock it is to be expected that the roots of all varieties will suffer about equal injury by low temperatures. This seems to be borne out by field observations; and yet trees of such tender varieties as Ben Davis, Tompkins King, and Twenty Ounce are more likely to die outright than are the trees of the relatively hardy varieties MacIntosh, Northern Spy, Greening, and even Baldwin. Two reasons for this are apparent. First, in the case of the tender variety, the direct injury is not restricted to the stock but in extreme cases may extend up the trunk to the crotch. Second, there is a tendency on the part of certain varieties to produce cion roots which are, in many cases, distinctly more hardy than the roots from the stock itself (2). These cion roots are found growing vigorously from MacIntosh trees, for example, on which the crab roots below are entirely dead. The possibility of an effect of the cion on root characters (3) deserves further study in this connection.

RELATION OF FUNGI TO INJURY

Although *H. sublateritium* is abundant in some localities on dead and dying wood of various trees, we have identified it on apple trees from only three sources. Two of these cases were in orchards in which only a single tree was involved. In the third orchard a block (Block 1) of 136 Baldwin trees about 50 years old has been under observation since May, 1925. At that time four trees had died in a localized area which appeared to be similar in soil type and drainage to other parts of the block. In September, 1927, two additional trees adjoining this area had died. *H. sublateritium*

was present on all of these six trees before they died and undoubtedly hastened their death. However, other trees in the same block showed severe injury at the crown and no evidence of the fungus; while, conversely, other trees bore an abundance of the mycelium in the outer cortex of the roots but showed little, if any, deeper injury and no apparent effect on the top. In inoculation experiments with this fungus (July, 1925) it has been found (November, 1927) to penetrate slowly through the outer cortex of large roots on old trees, but deeper penetration seems dependent on rather severe injury to the cambium and wood of the roots. In addition to the inoculations already described (4), on which the preceding notes were made, a single vigorous tree in Block 1 was inoculated by burying a rotting root in contact with a small wound on a large lateral root. At the end of the second growing season the mycelium could be found at the margin of the wound, but had not penetrated more than 5 millimeters in any direction.

This block of trees was mapped on June 4, 1926, and again on September 28, 1927, to show the number of trees injured at the crown and the number of injured trees which bore signs of the fungus. Of 46 injured trees of the first record, the fungus was found on 16. At the time of the last record the fungus was found on 12 out of 47 trees.

An attempt was made to inhibit the fruiting of *Hypholoma* by the application of powdered copper carbonate (100 and 200 gm.) or mercuric chloride solution (12 liters, 1–1000) around the bases of infected trees. After two seasons there was some fruiting following each of the treatments.

Occasionally other fungi of the mushroom type are found in a relation which resembles that of the *Hypholoma* as described above. However, these have not been found on more than an occasional tree in any orchard.

RELATION OF CULTURAL PRACTICES TO INJURY

The killing of roots and crowns of apple trees is profoundly influenced by the conditions which favor or retard the growth of the trees and the season of the year during which these conditions prevail. Thus a vigorous growth is of greatest importance in preventing injury; while an excessive growth or a late growth, which tend to prevent adequate ripening of the tissue in autumn and early winter, may prove dangerous.

The effect of cultivation on the degree of injury is well illustrated by two adjacent blocks of Baldwin (Block 2) and Wealthy (Block 3) which have been under observation since 1922. At that time Block 2 was in good cultural condition, and it has been cultivated every year except two (1925 and 1927) since that time. Block 3 had fallen into neglect before 1922 and has been cultivated only one season since that time. The soil of the two blocks appears to be similar in type and slope, and the trees are of about the

same age (15–20 years). Wealthy is considered by Chandler (1) to be very hardy, while Baldwin is only moderately so.

TABLE 1.—*Relation of tillage to root and crown injury of apple*

Block	Variety	Tillage	Number of trees				
			Total	July, 1922		September, 1927	
				Dead	Injured	Dead	Injured
2	Baldwin	Fair to good	208	1	2	5	8
3	Wealthy	Very poor	198	6	15	15	88

Table 1 shows the relative amounts of injury in the two blocks in 1922 and 1927. The variety Duchess had been grown as fillers in Block 3 up to 1926. In 1922 and again in 1925 this variety showed approximately three times as much injury as was found in the Wealthys.

While the emphasis here is placed on cultivation, observations indicate that applications of quickly available nitrogenous fertilizers have a similar effect in preventing injury or promoting recovery.

Faulty drainage in orchards has long been recognized as an important factor in bringing about the death of roots. However, only extreme cases have been given particular attention. It is becoming more apparent that a high water table or water-logged soil even for a short period of time may permanently damage the lower roots, thus weakening the tree and forcing the root system into a more exposed position (1). Orchards in which poor drainage appears to play an important part in bringing about root and crown injury are common in the principal fruit belt of western New York.

PREVENTION AND RECOVERY

Assuming that the orchard site has been selected, three things deserve special emphasis in preventing injury and in promoting the recovery of injured trees. The first two, adequate drainage and the maintenance of optimum growth conditions, have already been touched upon. The third, approach grafting of injured trees, has received more particular attention since 1923 and will be treated more in detail. Two types of grafts were used—ordinary apple seedlings such as are used in the nursery, and cion-rooted trees of the varieties MacIntosh and Delicious. Most of the grafts were made in 1924 and 1925. In September, 1927, 216 seedlings and 122 MacIntosh and Delicious grafts were examined in 6 orchards in western New York. Of these, 78 per cent of the seedlings and 81 per cent of the MacIntosh and Delicious grafts had made a good union. There was no significant difference between the MacIntosh and Delicious grafts. Growth

of all grafts varied widely with individuals, with soil conditions, and particularly with the condition of the tree on which the grafts were made. The treated trees ranged from slight injury to complete girdling at the crown. Trees with severe injury made fewer unions with the grafts, and the grafts which persisted made distinctly less growth on these trees. The maximum diameter attained by all grafts of 1924 and 1925 was 2 inches, with the average near 1 inch. The response of many treated trees is slower than was at first expected. In three orchards in which the trees are 40 to 60 years old it is yet too early to show how much benefit may be expected from this treatment. The grafts grow about as well on these old trees as on younger trees, but for obvious reasons the response of the trees is much slower. In one (Twenty Ounce) of the three younger orchards the conditions have been such as to produce additional injury on many trees. Most trees with severe injury have continued to decline in spite of treatment, and

TABLE 2.—*Terminal growth (in inches), over a five-year period, of an injured tree treated by approach grafting in 1923*

	Terminal number	1923	1924	1925	1926	1927
Least injured side	1	30.0	17.0	5.5	5.5	9.5
	2	17.0	14.0	5.5	5.5	11.5
	3	10.0	5.5	2.0	15.0	24.5
	4	10.0	1.0	5.5	6.0	19.0
	5	24.5	7.5	1.5	4.0	16.0
	6	3.5	1.0	4.5	7.5	8.5
	7	5.0	4.5	4.5	5.5	9.0
	Averages	14.2	7.2	4.1	7.0	14.0
Most injured side	8	3.0	0.7	1.0	0.7	0.7
	9	0.7	0.7	2.5	5.0	13.0
	10	0.7	0.5	0.5	2.0	9.5
	11	0.7	2.0	6.5	5.0	17.5
	12	3.0	1.0	7.5	3.5	17.0
	13	1.0	1.0	6.0	6.5	14.5
	14	1.5	6.0	8.0	9.5	12.0
	15	0.7	7.0	7.5	9.0	14.0
	16	1.0	1.0	5.5	6.5	20.0
	17	0.7	1.0	5.5	3.5	11.5
	18	1.5	1.0	8.0	6.5	16.0
	19	1.5	1.0	9.0	6.5	15.0
	20	1.0	2.5	2.5	9.0	9.0
	21	8.0	1.0	6.5	5.0	9.0
	Averages	1.7	1.8	5.4	5.5	12.7

it now seems that approach grafting in this orchard is of doubtful value until some of the adverse conditions, such as poor drainage, are altered. Even here, however, 80 per cent of 105 grafts have survived, and made a growth approaching that in other orchards.

In the two remaining orchards, mainly Wealthy and Greening, 2 of the 25 treated trees which were examined had blown over as a result of almost complete killing of the root system. The others with one exception were making a satisfactory recovery. An example of the best results which have been obtained from approach grafting is shown in table 2.

The measurements of annual growth of terminals were made on a Greening tree 8 to 10 years old on which at least three-fourths of the crown had been girdled and only one larger lateral root remained alive. Six seedlings were grafted into this tree in May, 1923. In September, 1927, four of these grafts were found measuring respectively 4, 3, 2.5, and 0.6 inches in diameter. It will be noted that little growth occurred on the weaker side of the tree during the first two seasons, and it may be argued that severely injured trees will not persist long enough to permit the grafts to become effective. On the contrary, many severely injured trees have been observed to survive without treatment for at least four or five years. Old trees are frequently seen with large dead areas at the crown dating back at least as far as the severe winter of 1917–1918.

SUMMARY

Additional evidence is offered to show that fungi are, at most, secondary agents in the production of root and crown injury in New York State.

The maintenance of optimum growth conditions including adequate drainage is emphasized for the prevention of injury and for promoting the recovery of injured trees.

For restoring injured trees the foregoing may be profitably supplemented by approach grafting. It is believed, however, that, except under the best orchard condtiions, trees more than half girdled are doubtful subjects for treatment.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

LITERATURE CITED

1. CHANDLER, W. H. Fruit growing. XV + 777 pp. Houghton Mifflin Company, Boston. 1925.
2. POTTER, G. F. Experiments on resistance of apple roots to low temperatures. New Hampshire Agr. Exp. Sta. Tech. Bul. 27. 1924.
3. SWARBRICK, T., and R. H. ROBERTS. The relation of scion variety to character of root growth in apple trees. Wis. Agr. Exp. Sta. Res. Bul. 78. 1927.
4. THOMAS, H. E. Root and crown injury of apple trees. N. Y. (Cornell) Agr. Exp. Sta. Bul. 448. 1926.

PHYTOPATHOLOGY

An International Journal
Official Organ of the American Phytopathological Society

EDITORS

E. C. STAKMAN, Editor-in-Chief, University of Minnesota, Univ. Farm, St. Paul, Minn.
H. M. QUANJER, Editor for Europe, Institut voor Phytopathologie, Wageningen, Holland
M. N. LEVINE, University of Minnesota, University Farm, St. Paul, Minn.
L. R. HESLER, University of Tennessee, Knoxville, Tenn.

ASSOCIATE EDITORS

W. B. TISDALE, Tobacco Experiment Station, Quincy, Fla.
MARGARET NEWTON, Manitoba Agricultural College, Winnipeg, Canada
DONALD FOLSOM, Maine Agricultural Experiment Station, Orono, Maine
S. M. ZELLER, Oregon Agricultural College, Corvallis, Oregon
FREEMAN WEISS, U. S. Department of Agriculture, Washington, D. C.
LESLIE COLEMAN, University of Toronto, Toronto, Canada
F. J. SCHNEIDERHAN, Virginia Polytechnic Institute, Blacksburg, Va.
C. W. BENNETT, Michigan Agricultural College, East Lansing, Mich.
M. W. GARDNER, Agricultural Experiment Station, Lafayette, Indiana
ANNIE RATHBUN GRAVATT, U. S. Department of Agriculture, Washington, D. C.
H. H. WHETZEL, New York State College of Agriculture, Ithaca, N. Y.
G. W. KEITT, Agricultural Experiment Station, Madison, Wis.

BUSINESS MANAGEMENT

R. J. HASKELL, Business Manager, U. S. Department of Agriculture, Washington, D. C.
J. F. ADAMS, Advertising Manager, Delaware Agricultural Experiment Station, Newark, Delaware

NOTICES

Subscription price. $5.00 per year United States and dependencies, Mexico and Cuba; Canada $5.25; other countries $5.50; current single numbers 50 cents. The journal is issued monthly beginning with Volume VIII, January, 1918.

Advertisements. Rates may be secured from the business management. No indorsement of any statements of claims made in advertisements is assumed by this journal or by the American Phytopathological Society.

Requests to supply copies of the journal must be made within 60 days from date of issue. Date of issue January 15 and monthly thereafter.

Separates. No gratis copies are supplied. A printed schedule of prices is submitted with the proof, and authors may secure separates by placing an order on the blanks provided when proof is returned.

Back Volumes with the exception of Vols. IV, V, and VI may be obtained unbound at $6.00 per volume, postage paid.

Separate copies will not be sold except in cases where the volumes are already broken. The price of such copies is $1.00 each for Volumes I to VII, inclusive, and 50 cents each for subsequent years. Prices net postpaid.

Manuscript may be sent to the nearest member of the editorial board. Clearness, brevity and conciseness are essential. In form and style, such as punctuation, spelling, and use of italics manuscripts should conform to the best usage in recent numbers of this journal. Manuscripts should be typed on one side of the paper, double spaced, and sent

unfolded. All papers longer than four printed pages should include a summary. The name and address of the institution at which the work was done should be included at the end of the summary. All footnotes should be numbered consecutively with arabic numerals except in tables, where small letters should be used. Each table should have a heading stating clearly and concisely its contents. References to literature should be made as footnotes only when four or less citations are given. The simplified method of citing literature used in recent numbers of this journal should be followed. References in "Literature Cited" should be listed alphabetically and numbered consecutively.

Illustrations necessarily must be limited in number, and photographs, to reproduce satisfactorily, must be of the best quality. Authors desiring unusual numbers of illustrations or special types of illustrations will be asked to bear part of the expense. All illustrations are reproduced on the same grade of paper and will be run as text figures wherever possible. Plates should be used only for a number of small illustrations which can not be run conveniently as individual text figures. Individual illustrations within a plate should be designated by letters to avoid confusion with text figures which are designated by arabic numerals. Authors should arrange plates to conform to page size and shape (4½ inches by 7 inches) after making due allowance for reductions. Specific reference to each illustration must be made in the text. There should be a complete legend for each illustration. All legends should be on separate paper and not on the illustration itself.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

Back Volumes may be obtained unbound at $6.00 per volume, postpaid, as follows: Volumes II, VIII, X, XI, XII, XIII, XIV, XV, XVI.

Separate copies of these and other volumes are available at $1.00 each for Volumes I to VII inclusive, and 50 cents each for subsequent years. Prices net postpaid.

American Phytopathological Society

OFFICERS

President

H. P. BARSS, Oregon Agricultural College, Corvallis, Oregon

Vice-President

F. D. HEALD, Washington Agricultural Experiment Station, Pullman, Wash.

Secretary-Treasurer

R. J. HASKELL, U. S. Department of Agriculture, Washington, D. C.

Councilors

C. L. SHEAR, U. S. Department of Agriculture, Washington, D. C.
F. D. FROMME, Agricultural Experiment Station, Blacksburg, Va.
E. C. STAKMAN, University of Minnesota, University Farm, St. Paul, Minn. (ex officio)
M. F. BARRUS, New York State College of Agriculture, Cornell University, Ithaca, N. Y. (ex officio)

Councilors Representing Divisions

DR. D. L. BAILEY, University of Toronto, Toronto, Ontario, Canada.
W. T. HORNE, University of California, Berkeley, Calif.
W. B. TISDALE, Tobacco Experiment Station, Quincy, Fla.